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BACTERIOLOGICAL TECHNIQUE AND
SPECIAL BACTERIOLOGY

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MANUAL
OF
BACTERIOLOGICAL TECHNIQUE
AND
SPECIAL BACTERIOLOGY

BY

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SECOND EDITION

ENLARGED AND THOROUGHLY REVISED

WITH NUMEROUS ORIGINAL ILLUSTRATIONS

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1902

PREFACE TO FIRST EDITION.

THIS Manual of Bacteriological Technique and Special Bacteriology has been written in pursuance of a plan first adopted by the writer while working in the Hygienic Institute, Berlin, and is the result of considerable experience of the wants of students in Bacteriology, both here and abroad. The technique and working methods have been carefully selected, and from the mass of available material on this rapidly growing branch of the subject, only those methods and material have been chosen which possess distinctive benefits. The methods are all straightforward and practical, and when carefully performed give excellent results.

It was thought best to separate the Technique from the Bacteriology of Special Diseases, which latter is included in the Special Bacteriology. This has been made very inclusive, and it is thought that it is quite sufficiently so, for any one not so specially equipped as to go to the original papers and articles. Much that is of importance in the study of the comparative diseases of animals and those transmissible to man has been included, an inclusion very necessary in the light of the growing importance of this to the practical hygienist. Again, to meet the needs of Sanitarians, an account has been given of the common bacteria found in water, milk, air, soil, etc. It is generally agreed that a book of this description is not a fitting place for original or controversial work, and that the object to be attained is the presenting of a correct idea of the relative conditions of the contemporary science—consequently little is alluded to of the above nature, and nothing speculative which could be avoided without interfering with inevitable deductions from fundamental experimental principles.

I take the opportunity of expressing my thanks to Dr George Nuttall, of the Hygienic Institute, Berlin, for many valuable suggestions, and for revising the manuscript of the technical portion of this work; to Professor Günther, of the same Institute, for the many pure cultures

he provided me with ; to Herr Hänsel, Manager for Carl Zeiss in Berlin, for his assistance in making the negatives reproduced by the colotype process in the four plates at the end of this work, also for valuable assistance in connection with the other illustrations ; to Dr M'Lean, Fortrose, N.B., for revising the proof sheets, and valuable suggestions regarding same.

I am also indebted to Herr Paul Altmann, 52 Luisen Strasse, and Carl Zeiss, 29 Dorotheen Strasse, Berlin, for the loan of the blocks used in illustrating the various Bacteriological Appliances.

THOS. BOWHILL.

BACTERIOLOGICAL LABORATORY,
29 CHAMBERS STREET,
EDINBURGH, *October 1898.*

PREFACE TO SECOND EDITION.

In presenting this second edition to bacteriological workers, the author feels that he has improved considerably upon the first edition. The work has been completely revised, in fact largely rewritten, and much new matter has been added both to the text and by way of original illustration.

The rapid sale of the first edition has given sufficient assurance of the usefulness of the book.

I have again to thank Herr Paul Altmann, Carl Zeiss, and also Messrs Charles Hearson & Co., for placing at my disposal the blocks used in illustrating the Bacteriological Appliances.

THOS. BOWHILL.

May 1902.

SUMMARY OF CONTENTS.

	PAGE
INTRODUCTION,	I
CLASSIFICATION AND MORPHOLOGY OF BACTERIA—	
Dimensions of Bacteria,	5
Flagella,	5
Spore Formation,	5
METHODS OF STERILIZATION—	
Sterilization by Dry Heat,	7
„ Moist Heat,	9
The Autoclave,	11
Sterilization of Post-mortem Instruments,	11

PART I.

PRINCIPLES OF BACTERIOLOGICAL TECHNIQUE—	
The Work-table,	13
General Methods of Bacteriological Investigation,	15
Preparation of Cover-glass Specimens,	17
Gram Method of Staining Cover-glass Specimens,	20
Bacteria that Stain according to the Gram Method,	21
The Claudius Method for Cover-glass Specimens,	21
Ziehl-Gabbet Method of Staining Tubercle Bacilli in Cover-glass Preparations,	22
Method of Staining Tubercle Bacilli in Cover-glass Specimens with Ehrlich's Anilin-water Stain,	23
Löffler's Method of Staining Glanders Bacilli in Cover-glass Specimens,	23
Methods of Staining Gonococci in Cover-glass Specimens,	24
Neisser's Method for the Differential Diagnosis of Diphtheria Bacilli in Cover-glass Specimens,	24
Unna's Method of Staining Fungi,	25

PRINCIPLES OF BACTERIOLOGICAL TECHNIQUE—continued:

	PAGE
Method of Examining Fungi Unstained,	25
Methods of Examining Milk for Bacteria in Cover-glass Specimens,	25
Microscopic Examination of Butter for Tubercle Bacilli,	26
" " Fæces " " 	27
Method of Staining Pneumococci in Pneumonic Sputum,	27
Methods of Staining Capsules of Bacteria,	28
" for Staining Flagella,	29
" of Staining Spores,	32
Method of Staining Yeast Spores,	34
" " the Nuclei of Yeast Cells,	34
The Examination of Blood in Cover-glass Specimens,	34
Stains for Malarial Parasites,	35
Method of Remounting and Restaining Cover-glass Specimens,	36
Necessary Precautions in Manipulating " "	36
Methods of Preparing Organs and Tissues for Bacteriological Examination, Freezing, Hardening, Embedding, and Section-cutting,	37
Staining Bacteria in Sections,	40
Stains,	50
Mordants,	54
Contrast-stains,	54
Methods of Cleaning Glassware,	56

PART II.

THE PREPARATION OF NUTRIENT MEDIA—

[illegible]

CONTENTS

ix

THE PREPARATION OF NUTRIENT MEDIA—*continued*:

	PAGE
Methods of Counting the Colonies which Develop in Cultures on Solid Media,	77
Quantitative Plate-culture Method,	79
Method of Obtaining a Pure Culture from a Colony on a Plate or Dish,	79
Methods of Cultivating Anaërobic Bacteria,	80
The Incubator,	83
Reactions Produced by Bacteria during their Growth,	88
Inoculation of Animals,	92
Observation of Animals after Inoculation,	95
Autopsies on Animals,	97
Methods of Examining Air,	99
" " Water,	102
" " Soil,	105
Method of Examining Unsound Meat,	106
" " Ice Cream,	106
" " a Diphtheritic Membrane,	107
Points to be Observed in Describing an Organism,	107

PART III.

SPECIAL BACTERIOLOGY—

Bacteria found in Inflammation and Suppuration,	109
The Streptothrices,	129
Bacteria Associated with Meat-poisoning,	155
Bacillus Orchiticus,	168
Epizoötic Lymphangitis,	169
Ulcerative Lymphangitis,	170
Bacillus Tuberculosis,	171
Pseudo-tuberculosis,	187
Bacillus Lepre,	189
Pseudo-tuberculosis Ovis,	190
Bacillus Smegmatis,	190
Spirillum Cholerae Asiaticæ,	191
" of Finkler and Prior,	196
" Tyrogenum,	197
Miller's Spirillum,	197
Spirochæte Obermeieri,	198
Bacillus Diphtheriæ,	199
" " Columbarum,	204
" " Vitulorum,	205
" Typhi Abdominalis,	205
" Coli Communis,	210

SPECIAL BACTERIOLOGY—*continued*:

	PAGE
Bacillus Equi Intestinalis,	212
„ Influenzæ,	212
Pseudo-influenza Bacillus,	215

THE SEPTICÆMIA HÆMORRHAGICA GROUP OF BACTERIA—

Bacillus Bovisepticus,	215
„ of Septic Pleuro-pneumonia of Calves,	216
„ Dysenteriæ Vitulorum,	216
„ Cholerae Columbarum,	217
„ of Duck Cholera,	217
„ Cholerae Gallinarum,	218
„ Phasiani Septicus,	219
„ of Grouse Disease,	220
„ of Canary-bird Septicæmia,	221
„ of Pneumo-pericarditis of the Turkey,	221
Pneumo-enteritis of Sheep,	222
Bacillus Felis Septicus,	223
„ Typhi Murium,	223
Swine Fever,	224
Bacillus Suisepcticus,	229
„ of Swine Erysipelas and Mouse Septicæmia,	230
The Bacillus of Bubonic Plague,	233
Broncho-pneumonia Bovis,	237
Bacillus of Canine Distemper,	238
„ Aërogenes Capsulatus,	241
Bacteria found in the Mouth,	242
„ „ Urine,	243
„ occurring in Air, Soil, and Water,	243
„ found in Milk,	252
„ causing Acetic Acid Fermentation,	258
„ „ Butyric Acid Fermentation,	259
„ „ Specific Changes in Beer, Wine, and Sugar,	260
The Phosphorescent Bacteria,	263
Thermophilic Bacteria,	264
Bacillus Capsulatus,	264
„ Megaterium,	265
Bacterium Zopfii,	265
Cladothrices,	266
Beggiatoa,	267
Bacteria found in Leguminous Nodules,	268
The Nitrifying Bacteria,	270

PART IV.

THE HYPHOMYCETES, OR MOULD FUNGI—

	PAGE
Achorion Schönleini,	272
Dermatomyces Gallinarum,	273
Trichophyton Tonsurans,	273
Thrush,	274
Oidium Lactis,	275
Penicillium Glaucum,	275
The Aspergilli,	276
The Mucors,	277
Fusisporium Moschatum,	278

PART V.

THE BLASTOMYCETES, OR YEASTS—

Saccharomyces Cerevisiæ, I.,	281
„ Ellipsoideus, I.,	282
„ „ II.,	283
„ Pastorianus, I.,	283
„ „ II., III.,	284
“Saccharomyces” Apiculatus,	285
Saccharomyces Anomalous,	285
„ Marxianus,	286
„ Membranæfaciens,	286
„ Exiguus,	286
„ Acidi Lactici,	286
Mycoderma Cerevisiæ et Vini,	286
The Torulæ,	287

PATHOGENIC BLASTOMYCETES—

“Saccharomyces Hominis,”	287
Saccharomyces Litogenes,	288
„ Neoformans,	288
„ Subcutaneus Tumefaciens,	288
“Blastomyces Dermatitidis,”	289

PART VI.

THE PROTOZOA—

Amœba Coli,	290
Balantidium (Paramœcium) Coli,	291

. THE PROTOZOA—*continued* :

	PAGE
Coccidia,	291
Coccidium Oviforme,	291
Klossia Soror,	292
Pyrosoma Bigeminum,	292
Endoglobular Parasites of the Dog,	295
Malarial Parasites,	296
Nagana, or Tse-tse Fly Disease,	299
Equine Syphilis,	300

PART VII.

DISEASES DUE TO INFECTIVE AGENTS OF UNDETERMINED CHARACTER—

Rabies,	301
-------------------	-----

DISEASES DUE TO UNDETERMINED INFECTIVE AGENTS WHICH PASS THROUGH BACTERIAL FILTERS—

Pleuro-pneumonia Contagiosa Bovis,	302
Foot-and-Mouth Disease,	303
African Horse-sickness,	305
Rinderpest,	309
INDEX,	315

LIST OF ILLUSTRATIONS.

FIG.	PAGE
1. Hot-air Sterilizer,	7
2. Koch's Steam Sterilizer,	7
3. Petri-dishes,	8
4. Bowhill's Steam Sterilizer,	9
5. Autoclave,	12
6. Nuttall's Microscope-Thermostat,	16
7. Hand Centrifuge,	26
8. Jung's Students' Microtome, with Freezing Attachment,	38
9. Schanze's Microtome " "	39
10. Koch's Apparatus for Solidifying Blood-serum,	68
11. " Plate-culture Levelling Apparatus,	72
12. Nuttall's Roll-culture Apparatus,	76
13. Wolffhügel's Counting Apparatus,	77
14. Esmarch's Apparatus for Counting Roll-culture Colonies,	78
15. Liborius's Tube for Anaërobic Cultures,	80
16. Kitasato's Flask for " "	81
17. Botkin's Apparatus for Anaërobic Plate-cultures,	81
18. Kipp's Hydrogen Apparatus,	82
19. Buchner's Tube for Anaërobic Cultures,	82
20. Hearson's Thermostat,	84
21. The Excelsior Gas Valve,	85
22. Hearson's Thermostat for Heating with Petroleum Lamp,	86
23. Incubator,	87
24. Smith's Fermentation Tube,	89
25. Dunbar's " "	89
26, 27. Reichel's Bacterial Filter,	91
28, 29. Cages for Inoculated Animals,	96
30. Hesse's Apparatus for Examining Air,	99
31. Air-pump for use in Petri's Method,	101
32. Slavo-Czaplewski Apparatus,	103
33. Fränkel's Earth-Borer,	105

FIG.		PAGE
34.	Staphylococcus Pyogenes Aureus,	To face 110
35.	Streptococcus Erysipelatos,	110
36.	Diplococcus Pneumoniæ,	118
37.	Bacillus Pneumoniæ,	118
38.	Schutz's Streptococcus of Strangles in Pus from Abscess,	122
39.	Micrococcus Tetragenus in Section of Spleen of Inoculated Mouse,	122
40.	Micrococcus Gonorrhœæ in Urethral Discharge,	124
41.	Actinomyces Bovis—Section of a Tumour from the Jaw,	130
42.	" " Agar Culture,	130
43.	Bacillus Anthracis—Virulent Bouillon-culture,	138
44.	" " with Capsules in Mouse's Blood,	138
45.	" " Stab-culture in Gelatine,	138
46.	" " in Blood of Mouse,	140
47.	" " Section of Mouse's Lung,	140
48.	" " showing commencing Involution Forms and Free Spores,	142
49.	" " containing Spores,	142
50.	" Edematis Maligni in Mesentery of Mouse,	144
51.	" " " Stab-culture in Gelatine,	144
52.	" Anthraxis Symptomatici, showing Flagella,	144
53.	" " " Stab-culture in Grape-sugar Gelatine,	146
54.	" " " and Spores,	146
55.	" Tetani—Stab-culture in Grape-sugar Agar,	146
56.	" " and Spores,	146
57.	" " Inoculation,	152
58.	" Proteus Vulgaris—Gelatine Plate,	152
59.	" Mallei—Section of Glanders Nodule from Horse,	152
60.	" Proteus Vulgaris, showing Flagella,	162
61.	" Mallei—Glycerine-agar Culture,	162
61a.	Glanders—Nasal Lesions in Horse,	162
62.	Bacillus Tuberculosis—Sputum,	172
63.	" " Glycerine-agar Culture,	172
64.	" " Pure Culture on Glycerine-agar,	172
65.	" " Lung of a Cow,	176
66.	" " Section of Horse's Mesenteric Gland,	176
67.	" " Section of Tubercle from Spleen of Pig,	182
68.	Scrofulous Gland from Neck of Pig,	182
69.	Bacillus Tuberculosis—Smear Preparation from Guinea-pig,	184
70.	" " (Avian)—"Branched Forms,"	184
71.	" Pseudo-tuberculosis—Smear Preparation from Nodule in Guinea-pig,	186

LIST OF ILLUSTRATIONS

XV

FIG.		PAGE
72.	Bacillus Lepræ—Section of Cutaneous Nodule,	<i>To face</i> 188
73.	" " " Affected Tissue,	" 188
74.	Spirillum Cholerae Asiaticae—Agar Culture,	" 190
75.	" " " with Flagella,	" 190
76.	" Finkler-Prior, with Flagella,	" 196
77.	Bacillus Diphtheriae—Blood-serum-agar Culture,	" 196
78.	" " Human Blood-serum-agar Culture,	" 200
79.	" Typhi Abdominalis—Gelatine Stab-culture,	" 200
80.	Avian Diphtheria—Chickens in the last Stages of the Disease,	" 200
81.	Bacillus Typhi Abdominalis—Agar Culture,	" 204
82.	" " " Section of Human Liver,	" 204
83.	" " " showing Flagella,	" 206
84.	" " " "	" 206
85.	" " " Agglutination Reaction,	" 208
86.	" " " " "	" 208
87.	" Coli Communis, with Flagella,	" 210
88.	" " " Gelatine Stab-culture,	" 210
89.	" " " Agar Culture,	" 210
90.	" of Fowl Cholera,	" 218
91.	" Phasiani Septicus—Agar Culture,	" 218
92.	" of Swine Fever—Spleen of Pig,	" 218
93.	" " " with Flagella,	" 224
94.	" " " Pure Culture from Pig's Lymph-gland,	" 224
95.	Stomach from Pig dead of Swine Fever, showing Ulcerations,	" 224
96.	Broncho-pneumonia Suis—Lung of Pig dead of Swine Fever,	" 224
97.	Bacillus Suisepiticus—Agar Culture,	" 228
98.	" Murisepticus in Mouse's Blood,	" 228
99.	" of Schweineseuche—Gelatine Stab-culture,	" 230
100.	" of Swine Fever—Gelatine Stab-culture,	" 230
101.	" " Erysipelas " "	" 230
102.	" of Mouse Septicæmia—Gelatine Stab-culture,	" 230
103.	" of Bubonic Plague—Oblique Agar Culture,	" 230
104.	" Pestis—Spleen of Inoculated Mouse,	" 234
105.	" " in Human Blood,	" 234
106.	" Subtilis,	" 246
107.	Spirillum Rubrum,	" 246
108.	Vibrio Rugula, with Flagella,	" 250
109.	Spirillum Undula " "	" 250
110.	Bacillus Megaterium and Spores from a Culture,	" 264
111.	Cladothrix found in Water,	" 264
112.	Nodule on the Root of Trifolium Pratense, showing Cells ; and (<i>b</i>) Bacteroidal Tissue,	" 268

FIG.		PAGE
113.	Nodule on the Root of <i>Trifolium Pratense</i> , showing Cell containing Bacteroids (<i>a</i>) ; and the Infection-thread (<i>b</i>),	<i>To face</i> 268
114.	Achorion Schönleinii—Agar Culture,	272
115.	„ „ Section through an Agar Culture,	272
116.	Trichophyton Tonsurans—Agar Culture,	272
117.	„ „ from Agar Culture,	274
118.	Oidium Lactis, from Gelatine Culture,	274
119.	Penicillium Glaucum „ „	276
120.	Aspergillus Niger, from Agar Culture,	276
121.	„ Fumigatus „ „	278
122.	Mucor Corymbifer, from Potato Culture,	278
123.	„ Mucedo, from Gelatine Culture, showing Zygotcs,	278
124.	Fusisporium Moschatum, from Potato Culture, showing Sickie-shaped Spores,	278
125.	Saccharomyces Cerevisiæ, showing Budding Cells—Potato Culture,	282
126.	„ „ Culture on Plaster Surface—(<i>a</i>) Ascospores,	282
127.	Coccidium Oviforme—from Liver of Rabbit,	290
128.	Section of a Rabbit's Liver, showing Coccidia,	290
129.	Klossia—Section of Liver of Snail,	292
130.	Pyrosoma Bigeminum in Blood of Ox,	294
131.	Young Ticks,	294
132.	Æstivo-autumnal Malarial Parasites of Man,	296
133.	Quartan Malarial Parasites of Man,	296
134.	Trypanosoma of Nagana (Tse-tse Fly Disease) in Blood of White Rat. × 400,	300
135.	Trypanosoma of Nagana (Tse-tse Fly Disease) in Blood of White Rat. × 1000,	300
136.	Contagious Pleuro-pneumonia of Cattle—Section of Lung of Cow,	302

INTRODUCTION.

THE micro-organisms causing putrefaction, fermentation, and infectious diseases belong partly to the lower vegetable, and partly to the lower animal kingdom, and are divided into four groups :—

1. *Hyphomycetes*, or *Mould Fungi*.
2. *Blastomycetes*, or *Yeast Fungi*.
3. *Schizomycetes*, or *Bacteria*.
4. *Protozoa*.

1. The *Hyphomycetes* consist of cells multiplying only by growth of the distal or point cells, and in this manner form threads or hyphæ. The fully-developed mould consists of a mycelium (which may be compared to the roots of higher plants), and of fruit hyphæ which develop out of the former, and bear on their extremities the spores or conidia. The moulds, in the absence of oxygen, frequently form cloudy mycelial masses in culture media. Spores are only formed on the surface of media in contact with oxygen. The *Hyphomycetes* possess no chlorophyll.

2. The *Blastomycetes* consist of ovoid or round cells, multiplying by bud-like processes from the mother cells, also at times by spore formation. There is reason to believe they are allied to the mould fungi. They likewise possess no chlorophyll. If the newly-formed cells are not detached, conglomerations of cells result. On unfavourable, strongly alkaline, and sugar-free media only a mycelium may develop.

3. The *Schizomycetes*, or *Bacteria*, comprise a large group of unicellular microscopic organisms, which multiply by a process of transverse division. They are spherical, oval, rod-like, or spiral in shape, and are generally devoid of chlorophyll, the green colouring matter possessed by the higher plants; owing to this circumstance they are forced to obtain their nutritive materials from organic substances, and therefore lead either a saprophytic or parasitic existence.

A *saprophyte* is an organism which obtains its nutrition from dead organic matter, whilst a *parasite* exists at the expense of some other living creature, known as its host. There is, however, a group of so-called "*facultative*" saprophytes and parasites which possess the power of accommodating themselves to different surroundings, at one time leading a parasitic, and at another time a saprophytic existence.

Decomposition, putrefaction, and fermentation result from the activities of saprophytic bacteria, whilst the parasitic bacteria cause changes in the tissues, resulting in disease processes, which may lead to the death of their host. It has been found convenient in classifying bacteria to describe their chief characteristics by the following terms :—

Chromogenic, for pigment-producing bacteria.

Photogenic, for phosphorescent or light-producing bacteria.

Zymogenic, for bacteria concerned in the various fermentations.

Saprogenic, for bacteria producing putrefaction.

Pyogenic, for bacteria producing suppuration.

Thiogenic, for those converting sulphuretted hydrogen into higher sulphur compounds.

There is another very important saprophytic group which comprises the so-called "nitrifying" and "denitrifying" bacteria. The "nitrifying" group oxidizing ammonia to nitrous and nitric acids; the "denitrifying" group reducing nitric acid to nitrous acid and ammonia. Through their association (symbiosis) with the nitrifying bacteria and the activity of the latter, leguminous plants are enabled to make up their nitrogen deficit in part from the free nitrogen of the air.

Owing to the absence of chlorophyll, bacteria must have the carbon and nitrogen necessary for their growth in the form of decomposable organic substances. The most favourable media for their development are neutral or very slightly alkaline solutions of proteid materials.

Bacteria growing, multiplying, and performing definite functions in the absence of oxygen, and to the existence of which oxygen is positively harmful, are known as "*anaërobic*" bacteria, in contradistinction to the *aërobic* group, for the proper performance of whose functions free oxygen is essential. Many organisms can accommodate themselves equally well to both these modes of existence—exposed to the air they are "*aërobic*," in its absence they become "*anaërobic*." Bacteria pos-

sessing this faculty are known as "*aëro-anaërobic*" or "*facultative*" organisms.

The most favourable temperature for the development of bacteria pathogenic for warm-blooded animals is that of the human body, viz., 37.5° C., while most of the saprophytic, including the so-called normal water bacteria, grow best at about 20° C. The "thermophilic" bacteria develop at temperatures ranging from 34° to 75° C. The range of the activity of bacterial growth depends upon specific differences and may have wide limits : some growing best at low, others at high, and others at medium temperatures. The minimum temperature is that temperature at which growth is just possible, the optimum when the growth is most luxurious, and the maximum the highest temperature at which the organism will develop.

Besides a suitable temperature, bacteria require for their development moisture and a medium of suitable composition and reaction.

Bacteria are also influenced to a varying degree by light—most forms develop by far the best in the dark. It is therefore important to have a dark closet in the laboratory for the storing of cultures. Cultures usually retain their vitality and virulence longer when maintained at low temperatures.

4. The *Protozoa* are unicellular animal organisms, usually considerably larger than the largest bacteria. Their protoplasm is differentiated into a homogeneous ectoplasm and a granular entoplasm which at times contains vacuoles, as also one or more nuclei. Some protozoa possess motile organs or flagella, others possess pseudopodia, others cilia. They multiply by fission or by spore formation. They may coalesce and form so-called plasmodia, and as in the bacteria the spores may be more resistant than the active organism. In some parasitic protozoa (malarial parasites, etc.) a true sexual process has recently been observed.

Classification and Morphology of Bacteria.

The following simple classification of bacteria has been found convenient by medical bacteriologists, though perhaps insufficient from a botanical point of view. The three principal divisions are as follows :—

1. *Micrococci* or *Cocci* : *spherical forms*.
2. *Bacilli* : *rod-like* "
3. *Spirilla* : *spiral* "

1. The *Micrococci* are subdivided into :—

Staphylococci—growing in grape-like clusters, the plane of division not being regular.

Streptococci—growing in chains, division occurring in one direction only.

Diplococci—growing in pairs.

Tetrads—growing in fours, dividing in two directions.

Sarcinæ—dividing in three directions, forming cubical bundles, of eight or more cells.

By *Ascococci* we understand cocci, associated in large numbers in an amorphous matrix, and enclosed in an enveloping membrane. When conjoined, micrococci may depart from the typical spherical form, being elongated or flattened, etc.

2. The *Bacilli* include all straight, rod-like bacteria, in which one diameter is greater than the other. Many of the organisms belonging to this group, in the course of development, deviate from the simple rod-shape, and when very short, *i.e.*, multiplying rapidly, may simulate micrococci. The ends of the rods also vary in shape according to the particular organism, being either blunt or rounded. Bacilli multiply by a process of division, which is transverse to their direction of growth. The mother-cell, having become elongated, divides into two daughter-cells—in this way chains of individuals are formed. The length of these chains varies in different species, and is also influenced by different conditions. Long chains of individuals, when this division is not very distinct, may, in unstained specimens, present a homogeneous, thread-like appearance. The rate of growth varies according to the species, nature of the medium, temperature, etc. The most rapid growth observed has been the division of a mother-cell into two daughter-cells within fifteen to twenty minutes.

3. The *Spirilla* may be subdivided into :—

Vibrios : short spirals.

Spirilla : long rigid spirals.

Spirochæte : long flexible spirals.

Different bacteria may, moreover, be characterised by the following peculiarities : They may form *Zoöglææ*, by which we understand agglomerations of large numbers of bacteria, enclosed in an amorphous matrix, which may give great tenacity to the bacterial mass.

Some bacteria possess a capsule which is more or less evident, depending upon the conditions of growth.

By *Leptothrix* we understand long undulating rods.

By *Cladothrix*, long, straight rods, showing pseudo-branching.

By *Streptothrix*, organisms that in their structure resemble at one time the thread fungi, and at other times the bacteria. A typical streptothrix occurs in the form of long irregularly branching threads, which may or may not show terminal swellings.

Involution-forms of bacteria usually occur under conditions unfavourable to the nutrition of the organism. Such forms are degenerative, and especially in old cultures may exhibit most eccentric shapes, bearing no resemblance to the normal organism.

DIMENSIONS OF BACTERIA.

The dimensions of bacteria vary as much as their form. The size is given in microns. A micron is the one-thousandth part of a millimetre, and is designated by the Greek letter μ . For example, *Bacillus anthracis* is 3-10 μ long, and 1-1.2 μ broad. *Bacillus tuberculosis* is 1.5-4 μ long, and 0.4 μ broad.

FLAGELLA.

All motile bacteria are provided with flagella, and it has been suggested that they should be distinguished, as in protozoa, according to the number and position of these motile organs, into:—

Monotricha, one flagellum at one pole.

Amphitricha, one flagellum at each pole.

Lophotricha, a bundle of flagella at one pole.

Peritricha, many flagella given off around the periphery of the organism.

The flagella are of varying length. They are invisible in unstained bacteria, and according to the stain used they may appear shorter or longer. Whereas they are usually wavy, the flagella of some of the spirilla are more or less rigid, resembling the cilia of protozoa.

(For special method of demonstrating flagella, see p. 29.)

SPORE FORMATION.

Certain bacilli develop what are known as *spores*, these bodies representing a resting stage in the life-cycle of an organism. Spores are

either formed within the mother-cells (endogenous), or certain individual cells prove more resistant. Endogenous spores are formed within bacilli, usually under conditions unfavourable to their growth—that is, when they have exhausted their food supply. Spores are usually resistant, at times highly so, to influences which destroy vegetative forms. They withstand drying, at times for many years, and great elevations of temperature, as well as the effect of chemical agents. Brought under favourable conditions the spores germinate, giving rise to the vegetative form or bacillus. Spores are not themselves directly capable of multiplication, but each spore produces a single bacillus which, dividing, gives rise to an indefinite number of individuals.

Spore formation is accompanied by the following phenomena :—

Bacilli about to proceed to spore-formation, as a rule, though not invariably, lose any motility they may possess. They seem to swell ; the protoplasm of the cell loses its normal homogeneous appearance ; granular refractive bodies of irregular shape and size are formed. These granular refractive bodies coalesce, the remainder of the cell remaining clear and transparent. Free granules may remain in the vicinity of the spore, as if not required for its development. The completely formed spore appears as an oval, highly refractive, glistening body, easily differentiated from the remainder of the cell, which now consists only of cell membrane and residual matter. Eventually, the cell membrane disappears, liberating the spore.

A single bacillus usually produces but one spore, which may be situated in the centre or at the extremity of the cell. The bacillus which produces a spore may assume the shape of a drum-stick or a spindle (known as a *Clostridium*). Spores cannot be stained by the ordinary methods employed for bacteria (for special methods of staining spores, see p. 32).

A spore about to develop into a bacillus gradually loses its highly refractive appearance, enlarges, and appears to assume a consistency approaching to that of the bacillus. The membrane of the spore is ruptured, and the bacillus grows out of the aperture. In some organisms the separation of the spore capsule is more evident than in others, and in this case the remains of the capsule may adhere for some time to the young bacillus.

It is on account of the resistance of the spores that the elaborate means of sterilization about to be described are necessary for obtaining media free from germs, by which in turn we can obtain pure culture

—that is, separate cultivations of single species of bacteria. The resistance of the spores is due to a very dense and impenetrable membrane, as well as to the fact that the protoplasm contains less water than that of the vegetative form. More water in conjunction with protoplasm lowers the temperature at which it coagulates.

Methods of Sterilization.

STERILIZATION BY DRY HEAT.

Sterilization may be accomplished by subjecting the articles to be treated to high temperatures, either in a moist or dry state. Successful sterilization by dry heat cannot usually be accomplished at a temperature lower than 150°C. , and the objects should be subjected to this temperature for not less than one hour. The apparatus used for hot-

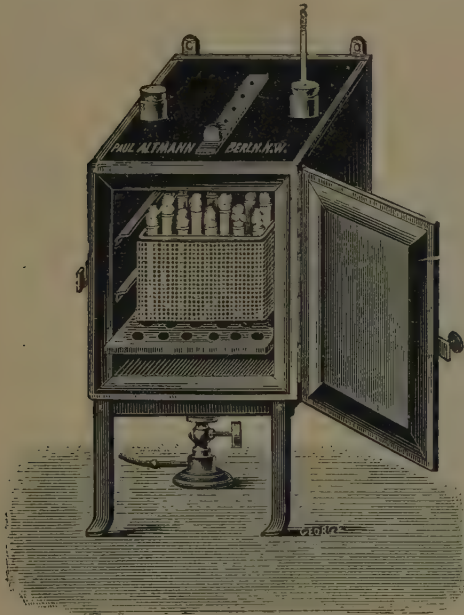


FIG. 1.—Hot-Air Sterilizer.



FIG. 2.—Koch's Steam Sterilizer.

air sterilization (Fig. 1) is double-jacketed, made of strong sheet-iron, and preferably with a copper bottom. Inside it is provided with two or more perforated movable shelves. On the top there is a regulating slide, by which a current of air through the apparatus may be secured, and two tubes, one for a thermometer and the other for a

thermo-regulator—which is usually not necessary—and two eyelets for suspending it to the wall, or it can be placed on a stand. When suspended against the wall, the latter should be protected by a sheet of asbestos. It is also desirable that the copper bottom of the apparatus should be detachable, thus enabling a new one to be fitted without difficulty.

In bacteriological work sterilization by dry heat is limited to such articles as glass flasks, plates, small dishes, test-tubes, pipettes, cotton wadding, and such metal instruments as are not seriously injured by the high temperature. The above articles are sterile when heated to 150° C. for one hour. The usual method is to remove the Bunsen burner when the thermometer registers 170° C., and then to allow the apparatus to cool before it is opened. If this is not done the glass-ware may crack from too rapid chilling.

Open vessels, before sterilization, are closed with cotton-wool plugs. Glass plates and pipettes are sterilized in copper or sheet-iron boxes specially manufactured for the purpose, and removed when required. It is convenient to wrap each pipette in paper.

Petri-dishes are most conveniently sterilized in a special cylindrical copper box with a capped lid, there being a round hole in the box and

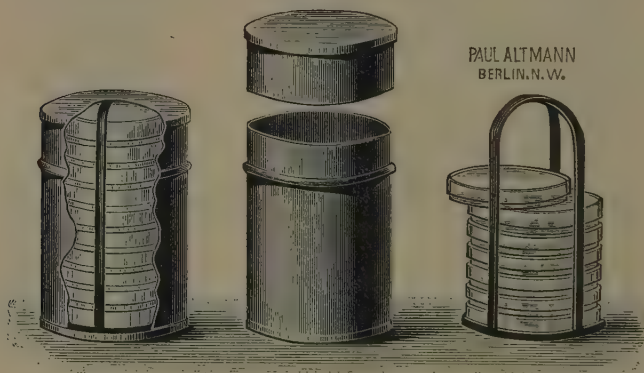


FIG. 3.—Petri-dishes.

in the lid. These holes are placed opposite each other during sterilization, thus allowing hot air to circulate in the interior of the cylinder. When the process of sterilization is completed, the lid is slightly turned, closing the holes. The interior of the cylinder is fitted so as to make it possible to withdraw one or any number of dishes when required (see Fig. 3).

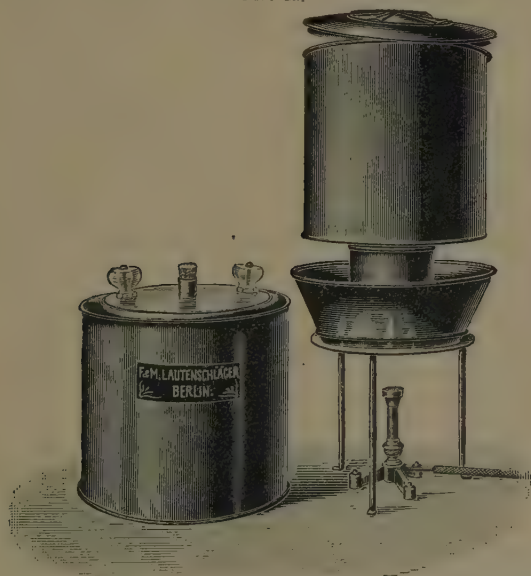
STERILIZATION BY MOIST HEAT.

Fluids, culture media, potatoes, etc., in fact anything that would be destroyed by the great heat of the hot-air chamber, are sterilized in the "*Koch Steam Sterilizer*" (Fig. 2), or the "*Arnold Steam Sterilizer*."

The Arnold apparatus possesses advantages over that of Koch. It is smaller but it heats more rapidly, whereas the Koch apparatus requires a long time to reach the temperature of 100° C., from which moment the period of sterilization should be determined.

Messrs Lautenschläger, of Berlin, have recently, at the writer's suggestion, constructed a useful portable steam sterilizer, costing twenty-seven shillings. Fig. 4a shows the apparatus with the outer cover removed, and the inner lid of the steam chamber slightly raised. The outer cover can also be used without the inner lid when a temperature lower than 100° C. is required; a place for a thermometer being provided.

FIG. 4a.



Bowhill's Modified Arnold Steam Sterilizer.

FIG. 4.

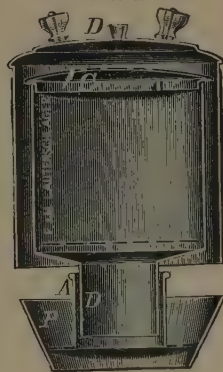


Fig. 4 shows a section of the apparatus, *P* being the water reservoir, the boiling chamber being constructed similarly to that of the Arnold apparatus. *A* and *D* show the junction of the steam

and water chambers by means of a strong detachable telescopic joint, enabling the water chamber to be cleansed, and also facilitating the packing of the apparatus during transportation. The size of the opening admitting the steam into the steam chamber is regulated by means of a diaphragm fitted on the inside of the steam chamber.

Novy has devised a simple sterilizer, the lower part of which consists of an ordinary water bath, 7 to 8 inches in diameter, the upper part being formed by a copper pail 8 inches high and 8 inches in diameter, with a perforated bottom and tubulated lid. Copper rings are soldered to the interior of the pail to prevent the tubes touching the sides. This prevents the cotton plugs from becoming saturated with the condensed steam which flows down the sides of the vessel. The pail is filled with flasks or tubes, and placed over the water bath, in which the water should be boiling. In five to seven minutes steam issues from the tube in the lid, showing that the interior temperature has reached 100°C . With the apparatus a student can attend to any needed steam sterilization at his own table, and thus save much time. The general usefulness of the apparatus, its compactness, cheapness, and the saving in gas, will recommend it to those practitioners who desire to equip a small laboratory.

A small steam sterilizer may be readily improvised by standing an inverted funnel of appropriate size upon the water bath. If the funnel is of glass it is well to surround it with a towel, to prevent its cooling too rapidly on removal. Dr Nuttall informs me that he has used this simple apparatus frequently during the last ten years and found it very useful.

To render a medium sterile, the process of sterilization may be either continuous or interrupted. Continuous sterilization is not usually employed, because it takes too much time. The spores which certain bacteria form are so highly resistant that they may withstand as much as six hours continuous boiling. Tyndall devised a method of discontinuous sterilization, which depends upon the principle that bacteria developing from spores in the nutrient media are destroyed by a relatively short exposure to a temperature of 100°C ., a temperature at which spores are not affected. To overcome the difficulty presented by the resistance of the spores, the tubes of media or other materials are placed in a steam sterilizer at 100°C . for thirty minutes. The first heating not having killed the spores, the material is placed at ordinary room temperature, or better, at 37°C ., when any living spores quickly

vegetate. The bacteria to which the spores give rise are killed by a second heating, for twenty or thirty minutes, on the following day. The above operation repeated three, four, or five times, ends in the certain sterilization of the media, all the spores having gradually developed into bacilli, and as such been destroyed.

The process of discontinuous or fractional sterilization at low temperatures is conducted in a somewhat similar manner to Tyndall's method, but requires a greater number of exposures to a temperature not exceeding 68° to 70° C., and is employed to sterilize materials, such as blood-serum, which would be rendered unfit for culture purposes by exposure to the temperature of steam.

THE AUTOCLAVE.

Whereas the above means of sterilization at 100° C. suffice for ordinary purposes, an apparatus for sterilization by steam under pressure, known as an *autoclave* (Fig. 5), is at times very useful, for the reason that the whole process of sterilization may be shortened from several hours to as many minutes. It can only be used, however, for media which remain uninjured by the exposure to the higher temperature obtained in the autoclave. The lid of the autoclave is held in place by a heavy horse-shoe shaped piece of metal (*h*) and the screw (*k*), which is tightened by means of the wrench shown in the figure. The weight (*a*) regulates the safety valve, whilst the manometer (*d*), which is provided with two needles, regulates the flow of gas and registers the pressure and temperature in the apparatus. The one needle is set at the point desired, the other, when the apparatus is heated, gradually moves up along the scale, until it reaches a point corresponding to that indicated by the first needle.

When an autoclave is used, a single exposure of fifteen minutes is sufficient to destroy all bacilli and their spores, when a pressure of one atmosphere is used. The autoclave must either remain closed until cool, or until the gauge indicates that pressure no longer exists, for if opened when the steam within is still under pressure, the steam will escape so rapidly that all fluids within the chamber thus suddenly relieved of pressure, will boil violently, and as a rule boil quite out of the tubes, blowing out the plugs.

STERILIZATION OF POST-MORTEM INSTRUMENTS.

Instruments may be sterilized by heating in a flame, boiling in strong soda solution, or placing them in the steam-sterilizer. Instru-

ments may be quite conveniently sterilized in the laboratory by dipping them in benzine and igniting the latter. The sterilization is thus com-

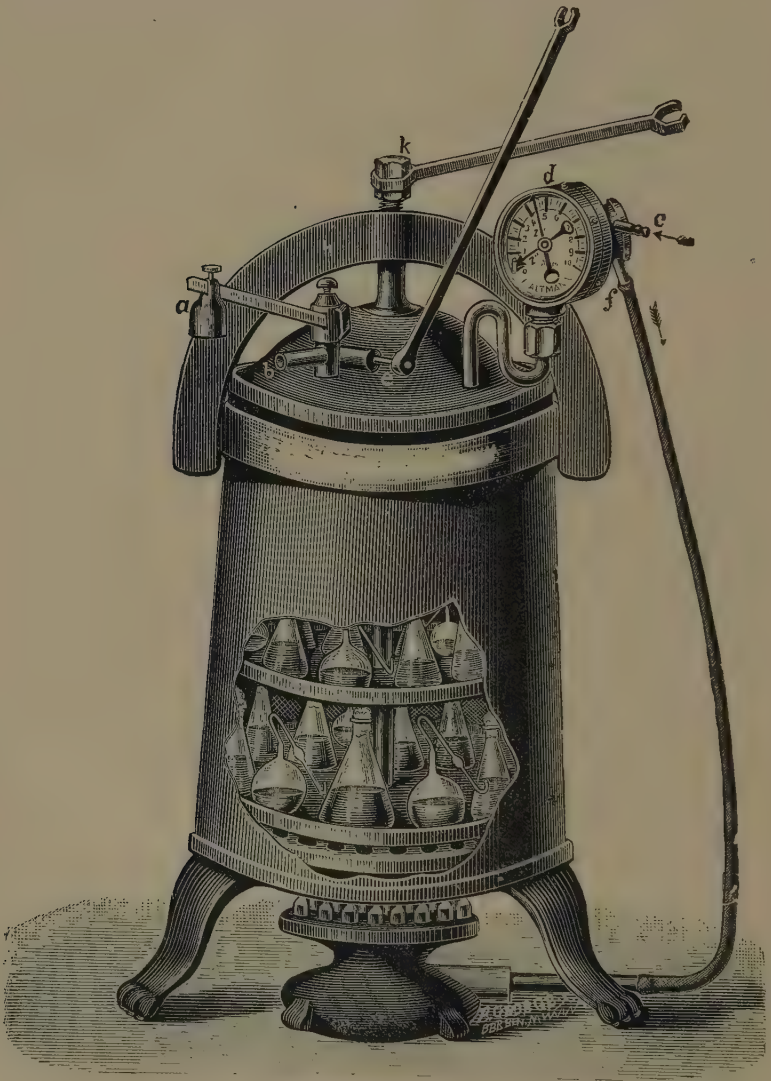


FIG. 5.—Autoclave.

pleted without exposing the instruments to as high a temperature as when they are drawn through the flame.

PART I.

Principles of Bacteriological Technique.

THE WORK-TABLE.

THE work-table must be horizontal and steady, suitable dimensions being 4 feet by 2 feet 4 inches on top, and 2 feet 4 inches high. The surface of the table may be covered with a piece of plain brown linoleum, which can be renewed when necessary, and forms an agreeable surface which resists the action of most chemicals. Piano-stools make excellent seats, through being adjustable for different heights. A sheet of plate or other thick glass is laid on the left hand side of the table, with pieces of black and white paper underneath, forming a background ; two pieces of coloured glass can also be used, one piece milk-white and the other black, according as the specimens under examination are stained or unstained. Under naked-eye examination unstained specimens or sections are best seen on the black, stained specimens on the white background. The black surface is most suitable for isolating the caseous portions of suspected (tuberculous) sputum.

Opposite and within easy reach of the worker a rack is placed to hold the bottles containing the stains and reagents in daily use. A solid block of wood with a number of circular holes bored in it is the most convenient rack. A Bunsen burner is placed on the right hand side of the table, but in the absence of gas a spirit lamp answers the purpose. On a shelf 3 or 4 feet above the right side of the table is placed a large bottle of distilled water, with a rubber tube attached to a syphon, or a tube opening at the bottom of the bottle and descending to a circular glass dish within easy reach of the worker. The extremity of the rubber tube is provided with a stop-cock. The water from the bottle is used for washing off excess stain, etc., from specimens.

The following articles are also placed on the table :—

1. A large glass dish (about 6×7 inches), with a cover, containing *Disinfecting Solution* (see p. 58), for the temporary disposal of old cultures, virulent material, etc.
2. A small glass dish ($3\frac{1}{2} \times 2$ inches), containing *Cleansing Solution* (see p. 57), for the temporary disposal of soiled slides and cover-glasses.
3. A covered glass dish, containing vaseline and a small camel's-hair brush, for preparing hanging-drop cultures.
4. One large test-tube stand.
5. One small test-tube stand.
6. One small filter stand.
7. One bottle of Canada balsam dissolved in xylol.
8. One small bottle of immersion oil.
9. Several ordinary glass tumblers, some of which are used for holding platinum needles and loops, clean water, scissors, forceps, glass rods, etc. A drawer is necessary in the table to hold clean cover-glasses, slides, test papers, filter paper, watch glasses, staining dishes, etc., when not in actual use.

The following stains and reagents are placed in the aforementioned rack for ordinary use :—

1. Löffler's solution of methylene-blue.
2. Watery alcoholic solution of gentian-violet.
3. Watery alcoholic solution of fuchsin.
4. Ziehl's carbol-fuchsin, or Ehrlich's anilin-water fuchsin.
5. Ehrlich's anilin-water gentian-violet.
6. Roux's double stain.
7. 2 per cent. acetic acid in water.
8. 3 per cent. hydrochloric acid alcohol.

Methylene-blue and gentian-violet give excellent results, and are useful stains for most bacteria, while fuchsin is one of the simplest and most rapid stains to manipulate. Ziehl's carbol-fuchsin is the most stable preparation of fuchsin, and is used in staining tubercle bacilli. Ehrlich's anilin-water-fuchsin and gentian-violet are very unstable, soon decomposing, and requiring to be freshly prepared every three weeks. Roux's double stain is specially adapted for the detection of diphtheria bacilli, inasmuch as the bacilli stain more readily and with greater intensity than any of the other organisms usually found associated with them. (For the preparation of the above stains and reagents, see p. 50.)

GENERAL METHODS OF BACTERIOLOGICAL INVESTIGATION.

THE MICROSCOPE.

For bacteriological investigation, a microscope with an Abbé condenser, iris-diaphragm, and low and high-power ($\frac{1}{12}$ immersion) lenses, is necessary. The following makers can be selected from :—

ZEISS.	LEITZ.
Objective AA.	Objective III.
" DD.	" VII.
Homogeneous Immersion, one-twelfth.	Homogeneous Immersion, one-twelfth.
Eye-pieces II. and IV.	Eye-pieces I. and III.

The low power is used with a narrow diaphragm for the examination of colonies of bacteria developed on plate or Petri-dish cultures.

Unstained specimens are always examined with a narrow diaphragm, concave reflector, and without an Abbé condenser, whilst stained specimens are examined without a diaphragm, with an Abbé condenser and a plane reflector. In examining double-stained sections an open diaphragm is used for the bacteria and a narrow diaphragm for the tissue. The oil-immersion lens after use must be cleaned with benzine, and wiped with a chamois-skin or special lens-paper. Excess of immersion-oil is removed from the cover-glass with xylol, which can also be used instead of benzine for cleaning the lenses.

THE HANGING-DROP.

1. Place directly in the middle of a clean cover-glass with the platinum loop (previously sterilized in the flame), a minute drop of the fluid to be examined.

2. The edges of the circular cavity in the hollow-ground slide are painted with a narrow strip of vaseline by means of a camel's hair brush.

3. Reverse the slide and place it upon the cover-glass, so that the inoculated drop is exactly in the centre of the hollow in the slide. Apply gentle pressure. The cover-glass adheres to the slide by means of the vaseline, forming an air-tight cavity. The preparation is now turned upwards quickly (*to prevent running of the drop*) and examined.

A convenient method for beginners, or those examining many drop-cultures, is that of Nuttall.* It consists in making small rings upon the cover-glass with a mixture of lamp-black and blood-serum applied with a fine camel's-hair brush, the cover-glass being held upon a turn-table.

* *Centralblatt f. Bakteriologie*, 1892, vol. xi., p. 539.

The dried ring is fixed in the usual manner by passing the cover-glass through the flame. The "drop" is placed within the ring, which renders it easy to bring the microscope to a focus upon the edge of the drop.

In examining a hanging-drop proceed as follows :—

1. Cut off the light coming through the condenser by narrowing the diaphragm to about the size of a pin's head.
2. Find the edge of the hanging-drop with the low power.
3. Regulate the Abbé condenser, and by manipulating the mirror cause the beam of light to fall upon the specimen.
4. Screw up the tube, remove the low power by shifting the nose-piece, and adjust the immersion-lens.
5. Increase the size of the diaphragm to about the size of a pea.
6. Put a drop of immersion-oil on the cover-glass.

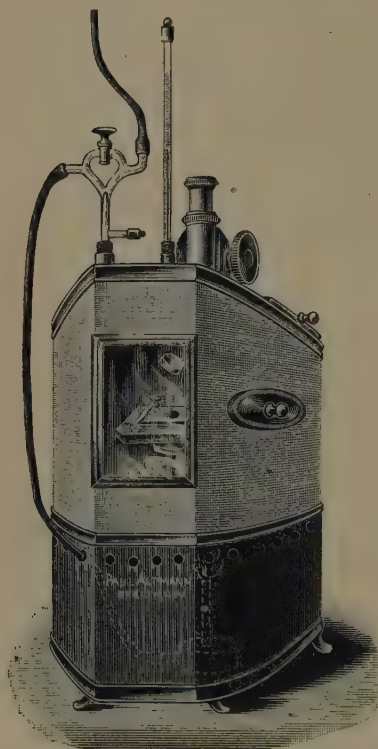


FIG. 6.—Nuttall's Microscope-Thermostat.

7. Screw down the tube with the coarse adjustment until the point of the lens touches the oil.
8. Screw down the tube carefully by means of the coarse adjustment until the object comes in view.
9. Regulate the focus with the fine adjustment.
10. If the field is not evenly illuminated, adjust the mirror without further removal.

The hanging-drop method is used to study living micro-organisms, their motility, processes of spore-formation, etc.

NUTTALL'S MICROSCOPE-THERMOSTAT.

This apparatus* is adapted for use with microscopes of different constructions, the instruments being placed in the thermostat from behind. The top of the apparatus, which is slanting, consists of two laterally movable slides, the inner

* *Centralblatt f. Bakteriologie*, 1895, vol. xviii., p. 330.

margins of which are made of removable strips of felt cut according to the shape of the microscope, so that the tube and adjustment remain outside. On the left side is an aperture, which can be closed by a hinged cap, and is large enough to admit the hand for the purpose of manipulating the slide. In front is a window admitting the light. On the right side screws connected with the mechanical stage can be adjusted; such an arrangement is, however, not usually necessary, and also increases the price. The thermostat is closed by a door at the back. This apparatus is useful for studying bacteria or protozoa in drop cultures, and has also been found useful in studying zoöparasites.

PREPARATION OF COVER-GLASS SPECIMENS.

ORDINARY METHOD.

1. Place a drop of sterile water on a clean cover-glass with a sterilized platinum-loop.
2. Inoculate the drop with a small quantity of the material under investigation—mix and spread well with the platinum-loop or needle.
3. Allow the material on the cover-glass to dry in the air.
4. Fix, by passing the cover-glass, preparation side uppermost, through the Bunsen flame three times, at intervals of one second, the cover-glass being held with "Cornet's Forceps."
5. Stain the specimen by flooding with the desired freshly-filtered stain, allowing the stain to act for from one to three minutes. (The staining process can be hastened and intensified by "heating," not "boiling," the cover-glass in the flame until vapour arises.)
6. Remove excess of stain by washing the specimen with the pipette, wash-bottle, or directly under the tap. (The specimen can be examined at this stage by laying it preparation-side downwards on a slide and removing excess of water with filter-paper. If of sufficient interest, it can afterwards be floated off and permanently mounted.)
7. Remove the excess of water by pressing the cover-glass gently between clean sheets of filter-paper, and allow it afterwards to become dry by exposure to the air or by waving it to and fro.
8. Mount in xylol-balsam.

Tissues may be examined for bacteria by means of "smear-preparations," the clean cover-glass being rubbed across the freshly-cut surface of the organ, or, a small piece of organ is held in the forceps and passed

lightly across the cover-glass or slide. Blood is best examined by placing a *small* drop in the centre of the (clean!) cover-glass, and spreading it by placing a second cover-glass upon the drop. The two cover-glasses are then quickly drawn apart by means of forceps, each cover-glass being held separately. The smears or films are allowed to dry in the air, and are then fixed in the flame and stained and mounted as above described.

Proceed as follows with the examination :—

1. Place a drop of immersion-oil on the centre of the cover-glass.
2. Screw down the tube with the coarse adjustment until the surface of the lens touches the oil.
3. Remove the diaphragm below the Abbé condenser, and arrange the flat mirror so that the field is illuminated.
4. Screw the tube carefully down with the coarse adjustment until the preparation comes in view.
5. Regulate the focus with the fine adjustment.
6. Obtain the desired illumination by manipulating the Abbé and mirror.

CONTACT, OR IMPRESSION SPECIMENS.

1. Lay a clean cover-glass gently on the top of the desired colony on the plate culture, apply gentle pressure, and lift the cover-glass up by one of its edges, avoiding lateral movement.
2. Dry by exposure to the air.
3. Fix in the flame, and proceed as with the ordinary cover-glass method, described above.

Preparations of colonies growing on slanted gelatine are obtained as follows :—

1. Remove the cotton-plug, and dip the tube in warm water, this will free the gelatine from the sides of the tube.
2. Slide the gelatine block out on a piece of cool glass or porcelain, and proceed as already described.

The above specimens differ from ordinary cover-glass preparations in that they present an impression of the organisms as they were arranged in the colony from which the preparation was made. It is important to note that liquefied-colonies cannot be used for the preparation of contact or impression specimens.

CZAPLEWSKI'S METHOD.

1. Place a few drops of carbol-gentian-violet (p. 29) on the fixed specimen, and heat gently over the flame for one minute.
2. Wash in water.
3. Place in Gram's iodine solution for thirty to sixty seconds.
4. Wash in water.
5. Wash in alcohol until no more stain comes away. If the stain dissolves imperfectly, add a few drops of anilin-xylol.
6. Wash in alcohol about one minute.
7. Wash in water.
8. Place in carbol-glycerine-fuchsin solution (p. 53), and warm slightly for about one minute.
9. Wash in water, dry, and mount in xylol balsam.

KISCHEWSKY'S QUICK METHOD.*

1. A drop of a weak solution of carbol-fuchsin (10 drops to 10 c.c. of water) is placed on a cover-glass and mixed with a minute quantity of the culture under investigation, and spread out in a thin layer.
2. The preparation is now heated gently over the flame, thereby becoming dried and fixed in a few seconds.
3. In examining blood, pus, etc., he recommends using carbol-fuchsin mixed with methylene-blue, manipulating in the same manner.

SEMENOWICZ AND MARZINOWSKY'S METHOD OF STAINING BACTERIA IN COVER-GLASS SPECIMENS AND SECTIONS.†

1. The cover-glass specimens are stained two minutes (sections three to four minutes) in a dilute solution of carbol-fuchsin (one part of a concentrated solution and two parts of water).
2. The specimens are now washed with water.
3. Stain the cover-glass specimens three to four minutes (sections four to five minutes) with Löffler's methylene-blue.
4. Wash in absolute alcohol.
5. Clarify in oil, transfer to xylol, and mount in balsam.

Results.—The carbol-fuchsin in the bacteria appears to work on the principle of a mordant to the methylene-blue. The nuclei and the

* *Centralbl. f. Bakteriöl.*, 1897, vol. xxi., p. 876.

† Baumgarten's *Jahresbericht*, 1897, p. 969.

bacteria are stained blue, the interstitial tissue and the protoplasm of the cells red, while degenerated bacteria also appear red.

GRAM METHOD OF STAINING COVER-GLASS SPECIMENS.

1. The fixed cover-glass specimen is stained two to five minutes with Ehrlich's anilin-water gentian-violet (see p. 51).
2. Wash with water.
3. Differentiate with Gram's solution of iodine (see p. 54) until the stained surface blackens like a tea-leaf—which usually takes a half to one minute.
4. Decolorize with alcohol until no more stain comes away.
5. Wash in water.
6. Dry, and mount in xylol-balsam.

The Gram method is useful for differential diagnosis, as some bacteria retain the stain and others do not.

According to Lehmann, the result depends very much upon the age of the culture and condition of the organism at the time the specimen is stained, as well as upon the method employed. The bacillus of black quarter, or "symptomatic anthrax," can also be stained under certain circumstances, although most authors make the contrary statement.

In order to check the Gram test and place results beyond doubt, proceed as follows :—

Take a clean cover-glass and put a small quantity of the material to be stained on one half, and on the other half a small quantity of a young culture of *Bacillus anthracis*. Air dry, fix, and stain by the ordinary Gram method above mentioned, so that both materials on the cover-glass are thus subject to the same reagents and conditions.

As the *Bacillus anthracis* stains readily according to the Gram method, one can judge whether the bacterial species under investigation stains or decolorizes by this method.

NICOLLE'S MODIFICATION OF THE GRAM METHOD.

1. Stain the fixed cover-glass specimen (one to five minutes) in the following solution, heating gently :—

Saturated solution of gentian-violet in 95 per	
cent. alcohol	10 c.c.
1 per cent. aqueous solution of carbolic acid	100 c.c.

2. Place in Gram's iodine solution for one minute.
3. Wash in absolute alcohol, plus one-third volume of acetone, until the specimen appears colourless.
4. Wash in water ; dry, or contrast-stain with safranin-solution with fuchsin or picro-carmin for two minutes.
5. Mount in xylol-balsam.

Bacillus anthracis.

Bacillus tuberculosis.

Bacillus lepræ.

Bacillus murisepticus	} Presumably varieties of the same species.
Bacillus erysipelatis suis.	

Bacillus tetani.

The Pyogenic Micrococci.

Micrococcus tetragenus.

Diplococcus pneumoniae (Fränkel).

Actinomyces hominis.†

Actinomyces bovis.†

Actinomyces musculorum suis

Botryomyces (Bollinger).

Discomyces (Rivolta).

Botryococcus ascoformans (Kitt).

Bacillus diphtheriæ.

Diplococcus intracellularis meningitidis.	} Only under certain circumstances.
Bacillus of Black Leg, or "Symptomatic Anthrax."	

Bacillus acidi lactici (Hueppe).

THE CLAUDIUS METHOD FOR COVER-GLASS SPECIMENS.†

Instead of using Lugol's solution, as in Gram's method, Claudius used picric acid.

By adding one-quarter per cent. solution of picric acid in water to 1 per cent. solution of methyl-violet in water, an indigo-blue colour is formed which is insoluble in water, very soluble in alcohol, chloroform, anilin-oil, and clove-oil.

* All the ordinary organisms not mentioned in this list decolorize by Gram's Method.

† At stage 4 during decolorization with alcohol the cover-glass must be kept moving, otherwise *Actinomyces* is almost entirely decolorized.

‡ *Annales de l'Institut Pasteur*, 1897, p. 332.

METHOD.

1. Place the material on a cover-glass and dry in the air.
2. Pass three times through the Bunsen flame.
3. Stain one minute in a 1 per cent. watery solution of methyl-violet.
4. Wash in water and dry with filter-paper.
5. Treat with picric acid solution * for one minute.
6. Wash in water ; dry with filter-paper.
7. Decolorize in chloroform or clove oil, and repeat the application until decolorization is complete.
8. Dry and mount in xylol-balsam.

Result.—The bacteria are stained a deep indigo-blue.

This stain has very little affinity for animal cells, their nuclei, etc., whilst it possesses the greatest affinity for certain bacteria. The bacteria stained by the Gram method also stain by this method, which in addition stains the bacillus of malignant oedema.

ZIEHL-GABBET METHOD OF STAINING TUBERCLE BACILLI
IN COVER-GLASS PREPARATIONS.

1. Spread a small portion of the material, sputum, caseous mass, mucus, etc., that is most likely to contain the bacilli, upon a clean cover-glass in as thin a layer as possible.
2. Air dry, and fix by passing three times through the Bunsen flame.
3. Place some Ziehl-Neelsen's carbol-fuchsin (see p. 51) in a watch-glass or small porcelain evaporating-dish, and float the cover-glass on the surface, film side downwards ; heat the stain until vapour arises, and set aside for one to two minutes. Another method is to flood the cover-glass with the stain, and heat three or four times until vapour arises.
4. Wash the cover-glass in water.
5. Place the cover-glass in Gabbet's solution (see p. 51) one to three minutes, according to the thickness of the preparation, and the intensity of the fuchsin stain.
6. Wash in water, dry.
7. Mount in xylol-balsam.

* Saturated solution of picric acid, to which an equal volume of distilled water has been added.

METHOD OF STAINING TUBERCLE BACILLI IN COVER-GLASS SPECIMENS WITH EHRLICH'S ANILIN-WATER STAIN.*

1. Prepare the material on the cover-glass in the same manner as described above.

2. Place some Ehrlich's anilin-water-fuchsin or gentian-violet (Ziehl's carbol-fuchsin can also be used) in a watch-glass or porcelain evaporating-dish, float the cover-glass on the surface, film side downwards (if it sinks it does not matter), heat the stain until vapour arises, and set aside one minute.

3. Withdraw the cover-glass with a small forceps, and wash off excess stain with water.

4. Lay the cover-glass, film side upwards, in a watch glass containing 3 per cent. HCl-alcohol (see p. 58) for one minute, and move to and fro, until it is decolorized.

5. Wash with water, and dry.

6. Contrast stain with a few drops of weak alcoholic or watery solution of methylene-blue or malachite-green for fuchsin stains, and Bismarck-brown for the violet stain, being careful not to stain long enough to obliterate any stained tubercle bacilli.

7. Wash in water, and dry.

8. Pass through the Bunsen flame. If passed many times through the flame the stained bacteria do not fade so readily. (Unna employs this method for leprosy bacilli.)

9. Mount in xylol-balsam.

LÖFFLER'S METHOD OF STAINING GLANDERS BACILLI IN COVER-GLASS SPECIMENS.

1. Make a smear-preparation from a young nodule, air dry, and fix.

2. Stain in Löffler's methylene-blue solution (see p. 51) five minutes.

3. Place the preparation one second in a 1 per cent. solution of acetic acid in water, which has previously been rendered a Rhine wine colour by the addition of a few drops of a watery solution of tropäolin OO.†

* Recommended by Günther.

† Through the addition of the tropäolin OO the cell plasma is entirely, and the nuclei partially decolorized, whilst the bacilli retain their colour.

4. Wash quickly with distilled water.
5. Dry, and mount in xylol-balsam.

METHODS OF STAINING GONOCOCCI IN COVER-GLASS SPECIMENS.

KNAACK'S METHOD.

1. Prepare a specimen from the pus in the ordinary manner, air dry, and fix.
2. Stain with methylene-blue.
3. Place the cover-glass in a 1 per cent. solution of argonin four minutes.
4. Wash and dry the specimen.
5. Place the cover-glass in a watery solution of fuchsin 1-20 for ten seconds.
6. Wash, dry, and mount in xylol-balsam.

NEISSER'S METHOD.

1. Place the fixed specimen in concentrated alcoholic solution of eosine, and heat gently for a few minutes.
 2. Dry the film with filter-paper and place immediately in a concentrated alcoholic solution of methylene-blue for fifteen seconds.
 3. Wash in water, dry, and mount in xylol-balsam.
- Result.*—The gonococci and cell-nuclei are stained blue, and the body of the cell red.

NEISSER'S METHOD FOR THE DIFFERENTIAL DIAGNOSIS OF DIPHTHERIA BACILLI IN COVER-GLASS SPECIMENS.*

1. Cover-glass specimens are prepared from cultures grown on Löffler's blood-serum, at 34° to 36° C.; the cultures must not be younger than nine and not over twenty-four hours' old; air dry, and fix the specimen in the flame.
2. Stain three seconds with Neisser's No. 1 solution (see p. 52).
3. Wash the specimen with water.
4. Stain three to five seconds with Neisser's No. 2 solution (see p. 52).
5. Wash in water, and examine; or dry, and mount in xylol-balsam.

Result.—The body of the diphtheria bacillus is faintly stained brown, and contains one to three blue granules, one at each end, or only one at one end, seldom any in the middle. The granules are oval and are never found free.

* *Zeitschrift f. Hygiene*, vol. xxiv., 1897.

UNNA'S METHOD OF STAINING FUNGI.

Solution *a*. Concentrated watery solution of methylene-blue.

„ *b*. Unna's glycerine-ether mixture.

1. Place the suspected material (crusts, etc.) on a slide, and saturate with acetic acid.
2. Crush the material with a second slide laid crosswise upon the first.
3. Heat over the flame until the acetic acid boils.
4. Separate the two slides and dry over the flame.
5. Remove fat with ether.
6. Flood with solution *a*, and heat over the flame until vapour arises.
7. Wash with water.
8. Decolorize in solution *b*, three or four seconds.
9. Wash with water.
10. Dry with filter-paper and over the flame.
11. Mount in xylol-balsam.

The fungi are stained a deep blue colour, while epithelial structures, owing to the glycerine-ether mixture, appear green. If this differentiation is not desired, the decolorization with glycerine-ether can be dispensed with.

METHOD OF EXAMINING FUNGI UNSTAINED.

1. Remove a little of the growth with a platinum hook on to a clean slide.
2. Add a few drops of a 1 to 5 per cent. solution of caustic potash, allowing it to act five to ten minutes (50 per cent. alcohol, containing a few drops of ammonia, can also be used).
3. Remove excess of potash with filter-paper.
4. Mount in glycerine-jelly.

METHODS OF EXAMINING MILK FOR BACTERIA IN COVER-GLASS SPECIMENS.

1. Dip a sterilized platinum-wire into the milk, and draw the wire sharply across the surface of a clean cover-glass.
2. Air dry, and fix in the flame.
3. Hold the cover-glass between the fingers, and flood the preparation side with some sulphuric-ether to remove the fat.

4. To demonstrate ordinary bacteria stain with methylene-blue, as it does not colour the background so intensely as fuchsin or gentian-violet. To demonstrate tubercle bacilli, stain the specimen

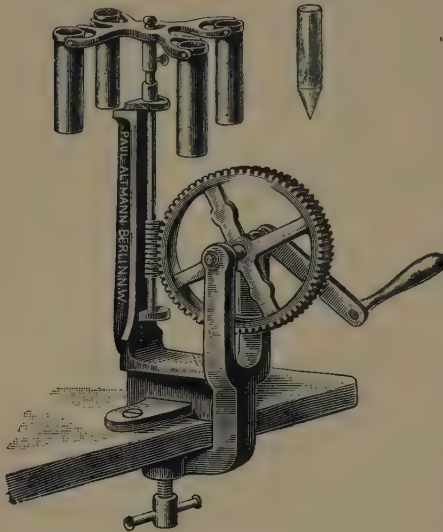


FIG. 7.—Hand Centrifuge.

according to the methods given on page 23. The centrifugal machine can also be used (Fig. 7), and the resulting sediment examined.

When few tubercle bacilli are present in milk or other fluids they are much more readily detected by the use of the centrifugal machine.

In the absence of a centrifugal apparatus, the following method will be found useful:—

1. To 200 c.c. of suspected milk add 10 c.c. of liquefied carbolic acid, and mix thoroughly.
2. Pour the carbolized milk into a conical urine-glass, and let it stand under cover for twenty-four hours.
3. Prepare specimens from the sediment, air dry, and fix in the flame.
4. Wash in equal parts of alcohol and ether, and dry with filter-paper.
5. Fix in the flame.
6. Stain with carbolic fuchsin.
7. Decolorize in 25 per cent. watery solution of hydrochloric acid, by dipping the specimen just long enough in the solution to remove the red colour.
8. Wash in 60 to 70 per cent. alcohol (the red colour reappears) until no more colour comes away. If desired, methylene-blue can be used as a contrast stain.
9. Wash in water, dry, and mount in xylol-balsam.

MICROSCOPIC EXAMINATION OF BUTTER FOR TUBERCLE BACILLI—ROTH'S METHOD.

1. About two to four grammes of the suspected butter are placed in a test-tube with a small spatula or a glass rod. The test-tube is then

filled about three-quarters full with water, placed in a water-bath at 50° C., and heated until the fat is thoroughly melted.

2. Cork the test-tube, and shake well a few times in order to separate any tubercle bacilli, etc., from the fatty substance.

3. Place the test-tube, bottom up, in the warm water-bath for fifteen minutes, until the fat is again thoroughly dissolved.

4. Place the test-tube in a cool place, so that the butter-fat again solidifies.

5. The fluid contents are then removed and either placed in the centrifugal apparatus or set aside until sediment forms.

6. Some of the sediment is placed on a cover-glass, air dried, slightly heated, laid in a mixture of ether and alcohol (1 to 3), removed, dried, and fixed in the flame.

7. The specimen is stained according to the ordinary process for tubercle bacilli.

For the isolation of ordinary bacteria from butter, the operation above described is conducted with proper precautions, sterilized water being mixed with the butter. The watery suspension is used for the inoculation of culture media, or if the butter is being examined for tubercle bacilli, the water is inoculated into guinea-pigs.

MICROSCOPIC EXAMINATION OF FÆCES FOR TUBERCLE BACILLI.

Rosenblatt* recommends the following procedure for finding tubercle bacilli in suspected cases of intestinal tuberculosis. Give the patient tinct. opii until the stools become hard. Scrape the surface of the fæces, or any mucopurulent secretion adhering thereto, and examine in the ordinary way. By this method bacilli situated on or immediately under the intestinal ulcerations adhere to the surface of the hard scybala, while they become lost in the volume of the ordinary liquid stools, where it may be difficult or impossible to find them.

METHOD OF STAINING PNEUMOCOCCI IN PNEUMONIC SPUTUM.

1. Prepare some cover-glass specimens, air dry, and fix in the flame. (If the material has been preserved with carbolic acid, wash the specimens in equal parts of alcohol and ether; dry, and pass again three times through the flame.)

* *C. f. inn. Med.*, 22nd July 1899, cited *Brit. Med. Jour.*, 13th Jan. 1900, p. 90.

2. Stain with anilin-water gentian-violet (p. 51) for fifteen to thirty minutes.
3. Wash in 0.6 per cent. NaCl. solution.
4. Place in Weigert's iodine solution (p. 42) for two to three minutes.
5. Dry and stain for about thirty seconds with a weak alcoholic solution of eosine.
6. Wash quickly in 0.6 per cent. NaCl. solution.
7. Dry and decolorize with Weigert's anilin-xytol solution (p. 58) till the specimen turns pink.
8. Wash with xylol, and mount in xylol-balsam.

METHODS OF STAINING CAPSULES OF BACTERIA.

ORDINARY METHOD.

Treat the fixed preparation as follows:—

1. Stain with 2 per cent. watery gentian-violet solution, and intensify the staining action by heating until vapour arises.
2. Wash with water, and dry.
3. Decolorize the capsules for six to ten seconds with 2 per cent. acetic acid in water.
4. Wash with water.
5. Mount preferably in water, as the high refractive index of balsam renders the capsules less visible.

Fuchsin can be used in place of gentian-violet. Many species of bacteria which were supposed not to possess a capsule show them very distinctly by this method. According to Johne, this method is of especial value in differentiating the capsule of *B. anthracis*.

FRIEDLÄNDER'S METHOD.

1. Lay the fixed preparation in 1 per cent. acetic acid one to three minutes.
2. Pour off the acid, and dry quickly.
3. Stain for a few seconds in anilin-water gentian-violet solution (p. 51).
4. Wash in water and examine. At this stage the capsules are sometimes so intensely stained that they obscure the enclosed bacteria.

5. Wash in 1 per cent. acetic acid or 50 per cent. alcohol for ten seconds.

6. Wash in water; examine, and if properly stained, dry and mount in xylol-balsam.

NICOLLE'S METHOD.

1. Stain the fixed preparation in the following solution:—

Saturated solution of gentian-violet in 95 per	
cent. alcohol	10 c.c.
Aqueous solution of carbolic acid (1 per cent.)	100 c.c.

2. Wash in absolute alcohol, plus one-third volume of acetone.

3. Wash in water; dry, and mount in xylol-balsam.

RIBBERT'S METHOD.

1. Stain the fixed preparation for a few seconds in the following solution:—

Distilled water	100 c.c.
Absolute alcohol	50 c.c.
Glacial acetic acid	12.5 c.c.
Dahlia	q.s. to dissolve by heating.

2. Wash in water and examine, or dry and mount in xylol-balsam.

KERN'S METHOD FOR DEMONSTRATING THE CAPSULES OF ANTHRAX BACILLI IN CULTURES.

1. Prepare a cover-glass specimen, air dry, and fix in the flame.

2. Place some anilin-water gentian-violet, or fuchsin, carbol-fuchsin, or Löffler's methylene-blue on the cover-glass, and heat 4 to 6 times at intervals of about one minute until vapour arises.

3. Wash with water.

4. Examine in water.

Capsules can be demonstrated on anthrax bacilli when cultures on agar, gelatine, and potato are used that are not more than two days old.

METHODS FOR STAINING FLAGELLA.

LÖFFLER'S METHOD,* MODIFIED BY GÜNTHER.

1. Take a young culture grown on slanted agar, make a hanging-

* *Centralbl. f. Bakteriol.*, 1889-90, vols. vi. and vii., pp. 209, 625.

drop specimen, examine it under the microscope, and note if the bacteria are motile.

2. Put a drop of distilled water on a clean cover-glass with a sterile platinum-loop, inoculate the drop with a little of the culture, and spread it gently.

3. Allow the specimen to dry in the air, and then pass it three times through the flame, but be careful not to heat too much.

4. Filter a few drops of Löffler's mordant (p. 54) on the cover-glass, and allow it to remain one-half to one minute. (Heat is unnecessary, as it only yields dirty, unsatisfactory specimens.)

5. Remove the mordant with a fine stream of water from the wash-bottle, and dry the cover-glass in the ordinary manner by blowing, etc.

6. Filter a drop of anilin-water fuchsin-solution on the surface of the cover-glass, or, without filtering, place a drop of fresh watery alcoholic solution of fuchsin on the cover-glass with a pipette, and heat the cover-glass over the flame until steam arises, remove, and allow the warm stain to remain one minute on the cover-glass, then wash off with water.

7. Dry quickly, and mount in xylol-balsam.

The addition of acids or alkali to the mordant, as originally recommended by Löffler, are now no longer considered necessary, and Günther considers heating the mordant unnecessary.

BOWHILL'S METHOD OF STAINING FLAGELLA AND BACTERIA SIMULTANEOUSLY WITH ORCEIN.*

1. A small quantity of material is taken from the surface of a young agar culture (previously observed to be motile), and a suspension made in boiled distilled water in a test-tube.

2. Leave the tube undisturbed for five minutes, then place one drop of the bacterial-suspension on a clean cover-glass, and air dry.

3. Fix in the flame, holding the specimen between the fingers to prevent excessive heating.

4. Pour some orcein solution (p. 53) in a watch-glass, float the cover-glass, preparation-side downwards, on the surface of the solution, and heat gently—*do not boil*—leaving the specimen in the solution ten to fifteen minutes.

5. Wash the preparation in the ordinary manner with water. Examine in water, and if satisfactory mount in xylol-balsam. The

* *Hygienische Rundschau*, 1898, vol. viii., pp. 11, 105.

advantage of examining the specimen in water is that the flagella appear more distinct than in balsam, and if too faintly stained, the specimen can again be placed in the orcein solution, and the process repeated.

VAN ERMENGEM'S METHOD.*

1. Prepare a cover-glass specimen from a suspension of a young culture in distilled water, air dry, fix in the flame, and place in the following solution—for thirty minutes if used cold, for five minutes if heated to 50-60° C.:—

Osmic acid solution (2 per cent.) . . . 1 part.

Tannic acid solution (10-25 per cent.) . . . 2 parts.

4 to 5 drops of acetic acid may be added to each 100 c.c. of the prepared solution.

2. Wash in several changes of distilled water.

3. Wash in absolute alcohol.

4. Place in a 0.5-0.25 per cent. solution of nitrate of silver for some seconds.

5. Without washing, place for some seconds in the following solution:—

Gallic acid 5.0 grammes.

Tannin 3.0 „

Fused acetate of soda 10.0 „

Distilled water 350.0 „

6. Repeat the steps 4 and 5, and keep the specimen moving in the solution (4) until the silverbath commences to turn black.

7. Wash in several changes of distilled water, and, if the specimen does not seem to be stained enough, repeat again from processes 4 and 5.

8. Dry with filter-paper and mount in xylol-balsam.

N.B.—The nitrate of silver solution must not be further used if precipitation occurs.

PITFIELD'S METHOD.

1. From a suspension of a young culture in distilled water, prepare some cover-glass specimens, air dry, and fix in the flame.

2. Cover the film with Pitfield's stain (p. 54), and gently steam over the flame on a copper section-lifter for one to two minutes.

3. Wash in water, dry, and mount in xylol-balsam.

* *Baumgarten's Jahresbericht*, 1893, p. 652.

FISHER'S METHOD.

1. From a suspension of a young culture in distilled water prepare some cover-glass specimens, air dry, and fix in the flame.
2. Stain by warming or boiling one minute in the following solution :—

Tannin	2 grammes.
Water	20 c.c.
• Solution of ferro sulphate	2 to 4 c.c.
Concentrated alcoholic solution of fuchsin	1 c.c.

3. Wash in water.
4. Stain with anilin-fuchsin, carbol-fuchsin, or saturated watery-solution of fuchsin.
5. Wash in water, dry, and mount in xylol-balsam.

BUNGE'S METHOD.

1. Prepare some cover-glass specimens as above (1).
2. To 5 c.c. of Bunge's mordant (p. 54) add about 14 drops of 3 per cent. solution of H_2O_2 (peroxide of hydrogen). Filter some of this solution on the cover-glass, heat gently, and allow the mordant to act for five minutes.
3. Wash in water.
4. Dry with filter-paper.
5. Stain the specimen in a solution of carbol-gentian-violet (p. 29), and heat gently during the process.
6. Place from one-half to one minute in a 1 per cent. solution of acetic acid. (This is not absolutely necessary.)
- 7. Wash in water, dry, and mount in xylol-balsam.

This method can be used for all bacteria. The capsules can also be stained at the same time if the specimen is placed in a 5 per cent. solution of acetic acid for one-half to one minute and washed in water before the mordant is applied.

METHODS OF STAINING SPORES.

Spores possess a firm membrane which shows great resistance towards staining reagents. Spores are best stained on cover-glass specimens.

ORDINARY METHOD.

1. Prepare the specimen in the ordinary way (p. 17), air dry, and fix in the flame.

2. Float the cover-glass, preparation-side downwards, on Ehrlich's anilin - water fuchsin or gentian - violet, or Ziehl's carbol-fuchsin, contained in a watch-glass or small porcelain evaporating-dish, and heated until vapour arises, when it is set aside to cool. Allow the stain to act for one minute. Repeat this process five times.

3. Decolorize with 3 per cent. HCl alcohol, allowing it to act one minute. The spores remain stained.

4. Wash the cover-glass with water.

5. Contrast-stain, with methylene-blue, if the spores are stained with fuchsin; if with gentian-violet, use Bismarck-brown as a contrast.

6. Wash in water, dry, and mount in xylol-balsam.

Result.—With *fuchsin* and *methylene-blue*, the spores are stained red and the bacilli blue; with *gentian-violet* and *Bismarck brown*, the spores are stained blue and the bacilli brown.

ANOTHER METHOD OF STAINING SPORES.

1. Prepare a cover-glass specimen, air dry, but do not fix in the flame.

2. Place the specimen in a slightly heated $\frac{1}{2}$ per cent. solution of hydrochloric acid in water.

3. Wash the specimen with water, and stain with carbol-fuchsin. The cover-glass held with Cornet's forceps is passed two to three times through the flame until vapour arises.

4. Decolorize with a 4 to 5 per cent. solution of sulphuric acid in water.

5. Wash with water, and contrast-stain with a solution of malachite-green.

6. Wash with water, dry, and mount in xylol-balsam.

METHOD OF STAINING THE SPORES OF *BACILLUS ANTHRACIS*.

1. Make a hanging-drop culture (p. 15) from the heart's blood of a mouse dead of anthrax.

2. Place the hanging-drop in an incubator from twenty-four to forty-eight hours, at 35° C., until spores are developed.

3. Remove the cover-glass from the slide.

4. Air dry.

5. Remove the vaseline with xylol.
6. Fix in the flame in ordinary way.
7. Proceed then as described above.

KLEIN'S METHOD OF STAINING SPORES.

1. Prepare an emulsion of the spore-containing material in normal salt solution, and to this add an equal quantity of filtered carbol-fuchsin solution.
2. Heat gently for about six minutes, holding the tube containing the emulsion a considerable distance above the flame.
3. Prepare cover-glass specimens from the emulsion, air dry, and fix by passing twice through the flame.
4. Decolorize with 1 to 2 per cent. solution of sulphuric acid in water about one to two seconds.
5. Wash in water.
6. Stain in a weak watery alcoholic solution of methylene-blue from three to four minutes.
7. Wash in water, dry, and mount in xylol-balsam.

METHOD OF STAINING YEAST SPORES.

1. Boil the prepared cover-glass specimen in carbol-fuchsin.
2. Decolorize in 4 per cent. sulphuric acid.
3. Wash in water and contrast-stain with methylene-blue.
4. Dry and mount in xylol-balsam.

METHOD OF STAINING THE NUCLEI OF YEAST CELLS.

1. Lay the prepared cover-glass specimen in a 3 to 4 per cent. solution of ferric ammonium sulphate for at least two hours.
2. Wash in water.
3. Stain for thirty minutes in a watery solution of hæmatoxinilin. (Make the solution with well- or spring-water.)
4. Wash thoroughly in water.
5. Differentiate one-half to two minutes in ferric ammonium sulphate solution, controlling the process by microscopic examination.

THE EXAMINATION OF BLOOD IN COVER-GLASS SPECIMENS.

1. Handle the cover-glass with forceps only.
2. Place a small drop of blood, about the size of a pin head, on the cover-glass, either directly from the living subject or with the platinum loop.

3. Spread the drop of blood by placing a second cover-glass on the top of the first. Avoid pressure, and draw the two cover-glasses apart. Another procedure consists in spreading the drop of blood obtained from the patient's finger with a piece of gutta-percha tissue or tissue paper across the cover-glass.

4. Air dry under a bell-jar.

5. The cover-glass can be fixed by either of the following methods :—

(a) Exposure to a temperature of 110-115° C. in the hot-air chamber for a few minutes.

(b) Immersion in absolute alcohol.

(c) Immersion in equal parts of absolute alcohol and sulphuric ether for thirty minutes.

(d) Immersion in a solution of formalin for five minutes.

(e) According to Ehrlich's method, on a heated metal plate.

The above methods of fixing are specially adapted for histological purposes.

6. To stain for bacteria use the ordinary stains. The special stains for malarial parasites are described below.

Precautions.—In examining specimens of blood for bacteria any excess of hæmoglobin should be removed before fixing, by placing the cover-glass specimen in a 3 per cent. solution of acetic acid for a few seconds, after which wash with distilled water, dry, stain, and mount according to the methods already described.

STAINS FOR MALARIAL PARASITES.

FUTCHER'S METHOD.

1. Fix the air-dried cover-glass specimen one minute in a 1 per cent. alcoholic solution of formalin, drain off the excess of fluid, and stain with the following solution :—

(a) Saturated solution of thionin in 50 per cent. alcohol, 1 part.

(b) 2 per cent. aqueous solution of carbolic acid . . . 5 parts.

2. Stain the specimen for thirty seconds, and wash with water. Dry with filter-paper and mount in xylol-balsam.

Result.—The red corpuscles are stained a light lavender, while the nuclei of the leucocytes and the malarial parasites are stained a purplish red.

ROMANOWSKY'S METHOD.

1. Dry the prepared cover-glass specimen rapidly over the flame, and then place it from three-quarters to one hour at 105° to 110° C.
 2. Stain from one to twenty-four hours with Romanowsky's stain (p. 53).
 3. Wash in water, dry, and mount in xylol-balsam.
- Romanowsky was able by this method to stain the nuclei of the parasites.

PLEHN'S METHOD.

1. Place the specimen for five to ten minutes in Plehn's stain (p. 52).
 2. Wash in water and dry.
 3. Mount in xylol balsam.
- Result.*—The blood corpuscles are stained red, the parasites blue.

METHOD OF REMOUNTING AND RESTAINING
COVER-GLASS SPECIMENS.

1. Heat the slide over the flame, and when the balsam is melted remove the cover-glass.
2. To restrain the cover-glass specimen, place it, after removal from the slide, in xylol for twenty-four hours. Renew the xylol several times to ensure thorough removal of the balsam.
3. The cover-glass is now placed in absolute alcohol to remove the xylol, and stained according to the method desired.

NECESSARY PRECAUTIONS IN MANIPULATING
COVER-GLASS SPECIMENS.

1. Never handle a heated cover-glass with cold forceps, as it generally breaks.
2. All specimens must be thoroughly air-dried before being fixed in the flame, otherwise the albumen coagulates, and the form of the bacteria is considerably affected.
3. During the process of fixing, the specimen must on no account be scorched in the flame, otherwise the form and staining properties of the bacteria, etc., are entirely lost.
4. To remove immersion-oil from the cover-glass of a freshly mounted specimen, absorb most of the oil with a piece of filter-paper, and remove the remainder with xylol when the balsam dries.

METHODS OF PREPARING ORGANS AND TISSUES FOR BACTERIOLOGICAL EXAMINATION. FREEZING, HARDENING, EMBEDDING, AND SECTION-CUTTING.

When the position of micro-organisms in organs and tissues and the histological changes they produce require to be studied, it is necessary to resort to section-cutting. To cut sections of the required degree of thinness, it is necessary to harden the organs. This is done either temporarily by freezing, or permanently by treating them with various hardening agents. In the latter case they may be cut directly as soon as they are hardened, or indirectly after first being embedded in some substance which fills the interstices of the tissue, and lends their otherwise friable structure support during the process of cutting.

FROZEN SECTIONS.

The cutting of frozen sections with the microtome is accomplished by means of an accessory apparatus sold with most modern microtomes, and consisting of an ether bottle, connected respectively with a rubber bulb and special platform for holding the block of tissue. The freezing is accomplished through the evaporation of the ether beneath the platform on which the substance to be frozen rests. A small microtome, manufactured by Jung of Heidelberg, known as the Students' Microtome, is adapted both for freezing and cutting sections embedded in paraffin, being fitted with a mechanical knife-guide, enabling the most inexperienced to make serial sections without difficulty (see Fig. 8) Fresh tissues may be cut directly after being frozen, or the freezing process can be applied to material preserved in alcohol; in the latter case all traces of alcohol must be removed from the hardened tissue before proceeding to section cutting:—

1. Place the pieces of tissue in a 1 per cent. solution of 40 per cent. formalin in water for two hours. Pieces of fresh tissue are prepared in a similar manner, or pieces of tissue removed from alcohol are dried with filter-paper and placed in aniseed-oil for at least twenty-four hours.
2. A piece of Joseph's paper or ordinary lens paper is laid on the freezing-plate of the microtome, a suitable piece of tissue is laid on top of it, and frozen with the ether spray. When hard it is cut into sections, and the cut sections are placed in water.

SECTIONS OF HARDENED TISSUES.

1. Cut the fresh organ or tissue into cubes of about half an inch.
2. Place the blocks of tissue on small pieces of filter-paper, write the name of the organ or tissue on the side of the paper, and place in alcohol, when the block of tissue becomes firmly fixed to the filter-paper.

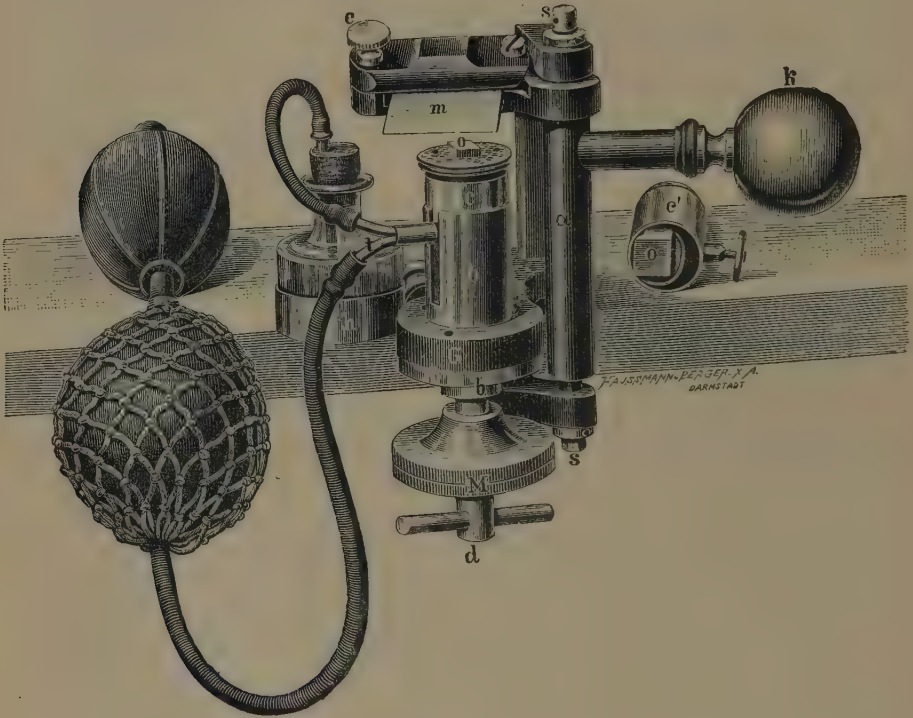


FIG. 8.—Jung's Students Microtome, with Freezing Attachment.

3. The blocks of tissue require two to three days to harden in alcohol, which should be changed several times.
4. Pieces of kidney, liver, and muscle, after remaining in alcohol two to three days, can be fastened to a block with a glue of the following composition :—

Gelatine	.	.	.	1 gramme.
Water	.	.	.	2 c.c.
Glycerine	.	.	.	4 c.c.

Heat and dissolve to a thick consistency, and glue the tissue to the block. Place in alcohol, and in twenty-four hours the gelatine will be sufficiently hard to admit of the tissue or organ being cut into sections with the microtome.

For cutting larger sections of tissue, or such as are embedded in celloidin, a microtome with the knife working at an angle is necessary. A most serviceable microtome is manufactured by Schanze of Leipzig (see Fig. 9).

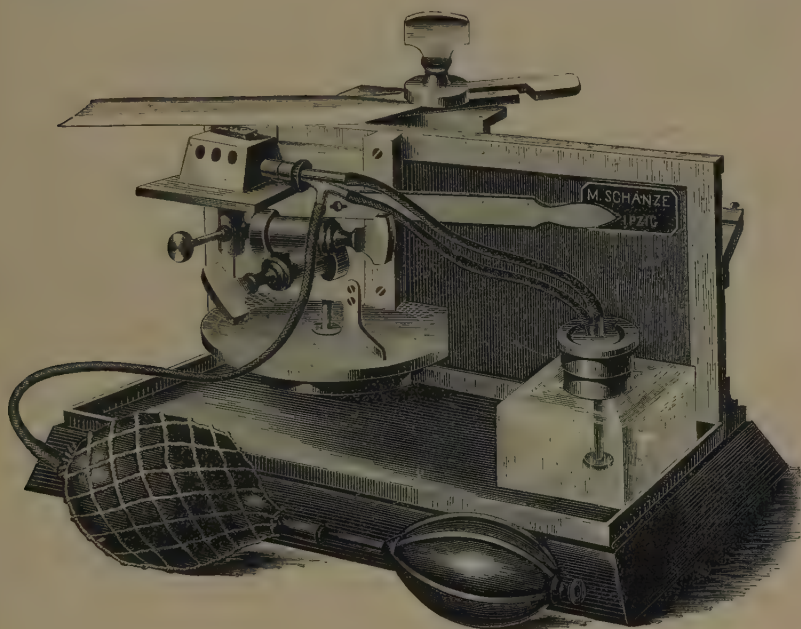


FIG. 9.—Schanze's Microtome, with Freezing Attachment.

It is important to remember that whatever microtome is used in cutting tissues embedded in celloidin, both the preparation and knife must be kept continually wetted with alcohol. The cut sections are removed from the knife with a camel's-hair brush, into 60 or 70 per cent. alcohol. It is absolutely necessary that the microtome-knife be sharp, the tissue well hardened and firmly attached to the block. In cutting sections of tissues for the demonstration of bacteria they need not be especially thin.

RAPID METHOD OF HARDENING AND EMBEDDING TISSUES IN PARAFFIN.

1. Place small pieces of the tissue in absolute alcohol, and change

the alcohol every half-hour, always using it in excess. In one and a half hours place the blocks of tissue in a small corked bottle of benzol until they become transparent (this takes a few minutes).

2. Pour off the benzol until the tissue is barely covered, then add a few shavings of paraffin, having a melting-point of 50° to 60° C., and place the bottle on the paraffin stove until the paraffin melts, then transfer the tissue to melted paraffin (50° to 60° C.) and allow it to remain for five minutes.

3. Pour the paraffin and tissue into a mould, and when the paraffin is set, cut in sections with a microtome and place the cut sections in warm water (50° to 60° C.).

EMBEDDING IN CELLOIDIN.

1. Transfer the hardened tissue or organ from the alcohol into equal parts of alcohol and sulphuric ether for twelve hours.

2. Transfer the tissue into a solution of celloidin made with equal parts of alcohol and sulphuric ether, and enough celloidin to form a thin syrup. Small pieces of tissue remain in this solution two days, while larger pieces remain four to five days.

3. Remove the piece of tissue and place it in a small porcelain evaporating-dish. Pour in some of the lower or thicker solution of celloidin until the tissue is covered. The dish is set aside until the celloidin is quite hard, at least twenty-four hours, sometimes longer being required.

4. Cut the celloidin away from the sides of the porcelain dish, place the embedded tissue on a block, and fasten with a little celloidin solution. Expose the block to the air until the celloidin hardens, which takes about two hours, and then place in 80 per cent. alcohol, and keep until required. Absolute alcohol must on no account be used, as it dissolves celloidin.

STAINING BACTERIA IN SECTIONS.

To stain bacteria in sections, the section is, with few exceptions, always brought directly out of the alcohol into the colouring solution. Sections remain longer in the dye than cover-glass specimens. It is often necessary to warm the staining solution either in the incubator or with a spirit-lamp or Bunsen burner, *but only till vapour arises*. Decolorizing processes have the effect of removing the stain from the nuclei and rendering them obscure; to prevent this, stain the sections

first with lithio- or picro-carmin. (See Günther's modification of the Gram method, p. 43).

To reduce or prevent the bleaching effects of alcohol during decolorization, add to the alcohol a small quantity of the stain with which the section was treated in the first place.

In clarifying sections do not use clove-oil for anilin dyes (except in the Claudius method), as it has the power of removing the stain. Use oil of cedar, or, better still, xylol, when dealing with bacteria.

WEIGERT'S METHOD (I.).

The section is carried from one watch-glass to the other with a needle or spatula.

1. Remove the section out of alcohol into water.
2. Stain with methylene-blue one to two minutes.
3. Wash the section in water.
4. Transfer to $\frac{1}{2}$ per cent. solution of acetic acid in water for one minute.
5. Transfer to absolute alcohol for thirty seconds, spreading the section out.
6. Place again in absolute alcohol for thirty seconds.
7. Into xylol thirty seconds.
8. Place on a slide, dry with filter-paper, and mount in *xylol-balsam*.

Anthrax bacilli are well demonstrated in sections by this method, as also many other bacteria, but care must be used not to carry the acid and alcohol treatment too far. The glanders bacilli can also be demonstrated in sections of tissue with this stain.

WEIGERT'S METHOD (II.).

1. Stain the sections five to ten minutes in a watery alcoholic solution of gentian-violet.
2. Wash in alcohol for five to ten seconds.
3. Wash in water for five to ten seconds.
4. Contrast-stain with Weigert's picro-carmin solution (p. 55) three to twelve hours.
5. Place in alcohol.
6. Clarify in Bergamot oil, oil of cedar, or xylol.
7. Mount in xylol-balsam.

Result.—The bacteria are stained blue, the nuclei of the cells red, and the surrounding tissue a bright yellowish red. Easily decolorized

bacteria, like *B. typhi abdominalis*, *B. mallei*, the bacillus of chicken cholera, etc., do not stain by this method.

WEIGERT'S STAIN FOR FIBRIN AND BACTERIA IN SECTIONS.

1. Harden the section in alcohol.
2. Stain in Ehrlich's anilin-water gentian-violet (p. 51) for five to fifteen minutes.
3. Wash the section in 6 per cent. sodium chloride solution.
4. Dry with filter-paper.
5. Place for two to three minutes in Weigert's iodine solution :—

Iodine	1 gramme.
Potassium iodide	2 grammes.
Distilled water	100 c.c.
6. Dry with filter-paper.
7. Decolorize in :—

Anilin oil	2 c.c.
Xylol	1 c.c.
8. Place the section in xylol to remove the anilin oil.
9. Mount in xylol-balsam.

LÖFFLER'S UNIVERSAL METHOD.

1. Bring the sections out of alcohol into Löffler's methylene-blue solution (p. 51) for five to thirty minutes.
2. Into 1 per cent. acetic acid.
3. Into absolute alcohol.
4. Into xylol.
5. Mount in xylol-balsam.

The amount of time the section remains in the acetic acid solution will depend on the species of organism. By this method bacilli are stained dark black-blue ; cellular constituents, blue ; protoplasm, light blue. This method can be employed for almost all bacteria.

KÜHNE'S METHYLENE-BLUE METHOD.

1. Bring the sections out of alcohol into Kühne's methylene-blue (p. 52) from one half to one hour (sections of leprous tissue should remain longer).
2. Wash quickly in water.
3. Wash in water containing 1.5 to 2 per cent. hydrochloric acid until the section becomes light blue in colour.
4. Transfer the section to a solution of lithium carbonate. (Con-

centrated watery solution of lithium carbonate, 6 to 8 drops ; water, 10 c.c.).

5. Place the section in clean water three to five minutes.
6. Immerse the section for a short time in absolute alcohol, to which a little methylene-blue in substance is added.
7. Rinse the section well in pure anilin oil.
8. Place the section in thymol or oil of turpentine for two minutes.
9. Place in xylol.
10. Mount in xylol-balsam.

The advantages of this method are that the bacteria remain stained, whereas the tissues are sufficiently decolorized to render the bacteria visible, and admit of the use of contrast-stains.

METHOD OF DOUBLE STAINING WITH METHYLENE-BLUE AND EOSIN.

1. Place the sections in alcohol for five minutes.
2. Place in Czinzinski's solution (p. 53) four to twelve hours.
3. Wash in water until the section acquires a pink colour and shows hardly any trace of blue.
4. Dehydrate in absolute alcohol.
5. Clarify in xylol, and mount in xylol-balsam.

Result.—The bacteria are stained blue and the tissues pink. The method can be employed for staining sections of diphtheritic membranes.

THE GRAM-GÜNTHER METHOD.

1. Remove the sections into water for two to three minutes.
2. Place in a solution of picro-carmin two to five minutes.
3. Wash the section in four to five changes of water.
4. Transfer to alcohol.
5. Transfer to Ehrlich's anilin-water gentian-violet, or methyl-violet (p. 51), one to two minutes.
6. Remove section into Gram's iodine-solution (p. 54) for two minutes, and spread the section out.
7. Place in alcohol thirty seconds.
8. Into 3 per cent. HCl alcohol (p. 58) for ten seconds.
9. Now dip the section many times into alcohol until no more stain comes away.
10. Into xylol one-half to one minute.

11. Remove the excess of xylol with filter-paper. Mount in xylol-balsam.

When the Gram method is used for sections without Günther's modification, only the bacteria are stained, but by first staining with picro-carminine a beautiful double-stained specimen is obtained, which shows distinctly the relation between the bacteria and the tissue. The Gram method does not stain the cells, except plasma or granule cells, which are sometimes mistaken for clusters of micrococci. Some of the epidermis cells also stain, and liver cells decolorize with difficulty. The same bacteria are stained in sections by Gram's method as are stained in cover-glass specimens.

NICOLLE'S MODIFICATION OF THE GRAM METHOD.

1. Stain the section first with picro-carminine (see Gram-Günther method, p. 43).

2. Stain the section for five minutes, as in processes Nos. 1, 2, and 3 in Nicolle's modification for cover-glass specimens (see p. 45).

3. Place the section for one to two seconds in 95 per cent. alcohol plus enough picric acid to colour it a greenish yellow.

4. Dehydrate in absolute alcohol.

5. Clarify in xylol or Bergamot oil.

6. Mount in xylol-balsam.

THE CLAUDIUS "CONTRAST-STAIN" FOR SECTIONS.

1. Stain the section with 1 per cent. watery solution of methyl-violet for two minutes.

2. Wash in water, and dry with filter-paper.

3. Place in picric acid solution (p. 55) for two minutes.

4. Decolorize in clove oil, and dry with filter-paper.

5. Place in xylol.

6. Mount in xylol-balsam.

Result.—The bacteria are stained blue, the tissues remaining yellow or colourless. Picro-carminine can also be used with this stain (for process, see Gram-Günther method, p. 43). This stain acts in a similar manner to Gram's, and for many bacteria it is preferable. For organisms stained by this process, see Gram's method, p. 21.

The original paper does not mention the method of applying the clove oil. In practice it is necessary to place the section on the slide

during the process of decolorizing with the clove oil, to prevent its curling up.

UNNA'S "DRY METHOD."

Many bacteria during the washing process become entirely decolorized, and to prevent this result the dry method of Unna may be employed as follows :—

1. The section is removed from the staining solution directly into water.
2. Spread the section out on a slide, and remove excess of water with filter-paper.
3. The slide must be carefully heated over the flame (not cooked) until dry, when it is allowed to cool and is mounted in xylol-balsam.

Contrast-stains are of little use in conjunction with this method, as the tissue-cells are too much altered by heating. Günther especially recommends this method as good for obtaining permanent specimens of tubercle-bacilli in sections.

NICOLLE'S METHYLENE-BLUE TANNIN METHOD.

1. Stain the sections in a watery alcoholic solution of methylene-blue (p. 51) for five to thirty minutes, or carbol. methylene-blue (p. 52) for thirty minutes.
2. Wash in water containing $\frac{1}{2}$ to 1 per cent. acetic acid for a few seconds.
3. Place the sections in 10 per cent. aqueous solution of tannic acid for a few seconds. (The methylene-blue becomes fixed and insoluble; 1 per cent. tannic acid solution is sometimes strong enough.)
4. Wash in water.
5. Dehydrate in alcohol.
6. Clarify in xylol or Bergamot oil.
7. Mount in xylol-balsam.

This method is recommended for staining easily decolorized bacteria, like the bacilli of typhoid fever, glanders, etc.

NICOLLE'S THIONIN METHOD.

1. Place the sections in carbol-thionin solution (p. 53) for one half to one minute.
2. Wash in water.
3. Dehydrate in absolute alcohol.
4. Clarify in xylol.

5. Mount in xylol-balsam.

This method is also used to stain easily decolorized bacteria.

R. PFEIFFER'S METHOD.

1. Stain the sections for fifteen to thirty minutes in the following solution :—

Ziehl's carbol-fuchsin (p. 51)	1 part.
Distilled water	3 parts.

2. Place in absolute alcohol, containing one to two drops of acetic acid to each watch-glass of alcohol, until the section acquires a reddish-violet colour.

3. Clarify in xylol or oil of cedar.

4. Mount in xylol-balsam.

MONEY'S METHOD.

1. Stain the sections in picro-carmine, borax-carmine, or alum-carmine (picro-carmine is preferable).

2. Place the sections in a watery alcoholic solution of gentian-violet or methylene-blue, adding to each watch-glassful of the stain used 2 to 3 drops of a 40 per cent. solution of formalin, and heat the stain until vapour arises.

3. Wash the sections in water.

4. Decolorize in 90 per cent. alcohol.

5. Clarify in xylol, and mount in xylol-balsam.

If the sections remain too long in the formalin solution, they decolorize with difficulty.

EHRlich's METHOD OF STAINING TUBERCLE BACILLI IN TISSUES.

(a) *Alcohol Sections.*

1. Place sections in Ehrlich's anilin-water fuchsin* (p. 51), and set the dish in the incubator at 37° C. for one hour and a half. If stained at room temperature, twenty-four hours are necessary.

2. Wash in water five minutes.

3. Transfer to 3 per cent. HCl alcohol one minute, and move the section about, do not allow it to simply lie in the alcohol.

4. Wash in water.

5. Contrast-stain with methylene-blue or Bismarck-brown.

6. Wash in water.

* Ehrlich's anilin-water gentian-violet can also be used, or Ziehl's carbol-fuchsin.

7. Into alcohol to remove water.
8. Into xylol.
9. Mount in xylol-balsam.

(b) *Frozen Sections.*

1. Transfer the sections from salt solution into 80 per cent. alcohol for five minutes to harden ; or,
2. Harden in corrosive sublimate, 1 : 1000-1500 for one-half to one hour, and wash in water, and proceed as above, No. 1 under "Alcohol Sections."

SPECIAL STAINS FOR THE GLANDERS BACILLUS.

Löffler's Method.

1. Stain the section in Löffler's methylene-blue (p. 51) for a few minutes.
2. Place the section in the following solution :—

Distilled water	10 c.c.
Concentrated sulphuric acid	2 drops.
5 per cent. solution of oxalic acid	1 drop.

for five seconds.

3. Decolorize and dehydrate in absolute alcohol.
4. Place the section in xylol.
5. Mount in xylol-balsam.

Another Method.

1. Transfer the sections from alcohol to distilled water.
2. Transfer to a slide, and dry thoroughly with filter-paper.
3. Stain thirty minutes with a few drops of :—

Carbol-fuchsin	10 c.c.
Distilled water	100 c.c.
4. Remove superfluous stain with filter-paper, wash the section three times with 3 per cent. acetic acid, not allowing the acid to act for more than ten seconds each time.
5. Remove all traces of acid with distilled water, after which absorb the water with filter-paper.
6. Dry the section with gentle heat according to the dry method of Unna (p. 45). Clear in xylol, and mount in xylol-balsam.

Unna's Method.

1. Dry the section on the slide and stain with Kühne's carbol-methylene-blue (p. 52) for ten minutes.

2. Wash in water.
3. Stain for fifteen minutes in a mixture of concentrated watery solution of tannic acid and 1 per cent. watery solution of acid fuchsin.
4. Dehydrate in alcohol.
5. Clarify in oil of Bergamot.
6. Mount in xylol-balsam.

Result.—The bacilli and cell-nuclei are stained blue, the tissue reddish.

METHOD FOR STAINING ACTINOMYCES IN SECTIONS.

1. Place the section on a clean slide or cover-glass, and stain in a 2 per cent. aqueous solution of rubin for two hours.
2. Wash in water until the section becomes dark red.
3. Wash quickly in 90 per cent. alcohol.
4. Wash in water until the section acquires a pink colour.
5. Stain in Löffler's methylene-blue for a half to one minute.
6. Wash in 90 per cent. alcohol until the sections are almost pink.
7. Clear in xylol, and mount in xylol-balsam.

Result.—The clubs of Actinomyces are stained red and the mycelium blue.

BUSSE'S METHOD OF STAINING YEAST-CELLS IN SECTIONS.

1. Remove the sections out of water and stain with hæmatein-alum solution (p. 55) for one-half to one hour.
2. Wash in water.
3. Contrast-stain with a very weak solution of carbol-fuchsin.
4. Dehydrate in absolute alcohol for five to ten minutes.
5. Clarify in Bergamot oil.
6. Mount in xylol-balsam.

Result.—The yeast cells are stained a brilliant red, and the tissue and cell-nuclei a dark bluish red.

METHODS FOR DEMONSTRATING CAPSULES OF BACTERIA IN SECTIONS.

Friedländer's Method.

1. Place the sections in the following solution, and keep at 37° C. for twenty-four hours:—

Concentrated alcoholic solution of gentian-violet	50 c.c.
Distilled water	100 c.c.
Acetic acid	10 c.c.

2. Differentiate with 1 per cent. acetic acid.
3. Dehydrate with alcohol.
4. Clarify in xylol or Bergamot oil.
5. Mount in xylol-balsam.

If process 2 is not applied long enough the capsules are as intensely stained as the bacteria, and obscure the latter.

Nicoll's Method.

Proceed as with Nicolle's method for staining cover-glass specimens (p. 29) ; but after process 3 dehydrate in absolute alcohol, and clarify in xylol or Bergamot oil. Mount in xylol-balsam.

GENERAL PRECAUTIONS TO BE OBSERVED IN THE MANIPULATION AND
STAINING OF SECTIONS.

1. When staining sections make a preliminary trial of the method before attempting to stain any considerable number of specimens.
2. In the various staining methods above described precise directions as to time, etc., have been given ; it is well, however, to remember that these directions are subject to variation under different conditions, a great deal depending on the ingenuity and perseverance of the individual operator in the attainment of good results, the method being modified to suit the case.

The following are the most common Errors in Staining Technique.

1. Portions of organs left too long before being examined or put in alcohol commence to putrefy, *i.e.*, putrefactive bacteria and fungi gain access, and in staining sections from such a specimen, for a definite organism, the putrefactive bacteria are also stained, and such being the case, particular attention must be given to the distribution of the bacteria throughout the section, as putrefactive bacteria penetrate the organ or piece of tissue from without, their numbers are therefore found to diminish in proportion to the distance from the outside surface, while the inner portions are usually quite free from putrefactive bacteria.

2. Many staining solutions, *i.e.*, carmine, hæmotoxilin, and some of the anilin staining solutions, often contain bacteria.

3. Faulty staining is liable to occur by the Gram method, when

the colouring matter is deposited on the surface of the sections. The use of too strong acids sometimes causes bacilli to break up into beads, when they are liable to be mistaken for a chain of cocci. In tissues, it is necessary to remember that plasma or granule cells often resemble colonies of micrococci. They are, however, distinguished by the granules being of unequal size.

STAINS.

CONCENTRATED ALCOHOLIC SOLUTIONS (STOCK).

Fuchsin	10 grammes	} dissolved in 100 c.c. alcohol,
Gentian-violet	7 "	
Methyl-violet	5 "	
Methylene-blue	5 "	

or take about half an ounce of each dye, and place in 8-ounce bottles filled with alcohol and provided with glass stoppers. These solutions are not directly employed for staining.

STOCK SOLUTION OF SAFRANIN.

Safranin	1 gramme.
Alcohol	50 c.c.
Water	50 c.c.

Before using mix one part of the solution with five parts water, and stain the specimen two to three minutes.

FLUORESCEIN.

This stain is used in saturated alcoholic solution.

ORDINARY STAINS FOR DAILY USE.

Method I. Mix 5 c.c. of concentrated alcoholic solution (stock, see above) of the desired dye with 50 c.c. of distilled water.

Method II. Add 2 grammes of undissolved anilin dye to 85 c.c. of distilled water. Boil five to ten minutes, and, after cooling, add 15 c.c. of 90 per cent. alcohol. Mix thoroughly and filter.

Method III. Fill an ordinary test-tube three-quarters full of distilled water, and add concentrated alcoholic or watery solution of the dye, until you can just see through the solution, which is then ready for use.

SPECIAL STAINS FOR COVER-GLASS SPECIMENS AND SECTIONS
OF TISSUES.

Ziehl's Carbol-fuchsin.

Fuchsin	.	.	.	1.0 gramme.
Carbolic acid	.	.	.	5.0 grammes.
Alcohol	.	.	.	10.0 c.c.
Distilled water	.	.	.	100 c.c.

GABBET'S SOLUTION.

Methylene-blue	.	.	.	1 to 2 grammes.
Distilled water	.	.	.	75 c.c.
Concentrated sulphuric acid	.	.	.	25 c.c.

This solution is used in the Ziehl-Gabbet method for demonstrating the presence of the bacillus of tuberculosis; the acid decolorizes, whilst the methylene-blue acts as a contrast-stain, and is of especial value owing to the simplicity and rapidity of its action.

ROUX'S DOUBLE STAIN.

Dahlia or gentian-violet	.	.	.	0.5 gramme.
Methyl-green	.	.	.	1.5 grammes.
Distilled water	.	.	.	200 c.c.

When staining cover-glass specimens proceed as follows:—

1. Air-dry and fix.
2. Stain five to ten seconds.
3. Wash in water.
4. Dry, and mount in xylol-balsam. Sections remain twelve hours in this stain; they are then washed, dehydrated, and mounted. This stain is of especial value in the examination of diphtheritic specimens.

LÖFFLER'S METHYLENE-BLUE.

Concentrated alcoholic solution of methylene-blue	.	.	.	30 c.c.
Watery solution of caustic potash (1 : 10,000)	.	.	.	100 c.c.

This stain is of very general utility. It was first used for staining the glanders bacillus.

EHRlich's ANILIN-WATER GENTIAN-VIOLET, FUCHSIN, OR
METHYL-VIOLET.

Anilin oil	.	.	.	4 c.c.	} Known as "anilin-water."
Distilled water	.	.	.	100 c.c.	

Shake the above well together, filter, and add 11 c.c. of a concentrated alcoholic solution of gentian-violet, methyl-violet, or fuchsin, according to the stain required. Shake the mixture, and set it aside for twelve to twenty-four hours before using.

These stains yield good results with many forms of bacteria, especially tubercle bacilli. They may be used in conjunction with Gram's method, but they have the drawback of being unstable, requiring to be renewed about every three weeks.

KÜHNE'S METHYLENE-BLUE.

Methylene-blue	1.5 gramme.
Absolute alcohol	10.0 grammes.
5 per cent. carbolic acid solution in water	100.0 c.c.

Mix the methylene-blue and alcohol; rub in a mortar until the blue is thoroughly dissolved, and then add 5 per cent. carbolic acid solution.

CHLOROFORM-FUCHSIN SOLUTION.

One or two crystals of fuchsin are dissolved in 2 to 3 c.c. of chloroform. Or else use concentrated alcoholic solution of fuchsin 2 to 3 c.c., to which 3 to 4 drops of chloroform have been added.

This method was devised by Aron for staining tubercle bacilli in fatty substances, milk, etc.

NEISSER'S STAINS FOR DIFFERENTIAL DIAGNOSIS OF THE DIPHTHERIA BACILLUS.

I.

Methylene-blue (Grübler)	1 gramme.
96 per cent. alcohol	20 c.c.

When dissolved, add—

Distilled water	950 c.c.
Acetic acid	50 c.c.

II.

Vesuvium	2 grammes.
Boiling distilled water	1000 c.c.

Allow this solution to cool after filtration.

PLEHN'S STAIN FOR MALARIAL PARASITES.

Concentrated watery solution of methylene-blue	60 parts.
0.5 per cent. eosin solution in 75 per cent. alcohol	20 "
Distilled water	40 "

ROMANOWSKY'S STAIN FOR MALARIAL PARASITES.

Saturated aqueous solution of (old) methylene-blue 2 parts.
 1 per cent. aqueous solution of eosin 5 parts.

Before using, remove the film that forms on the surface of the solution, to avoid a crystalline deposit on the specimen. The success of the method largely depends upon the particular brand of methylene-blue used.

CZAPLEWSKI'S CARBOL-GLYCERINE-FUCHSIN.

Fuchsin	1 gramme.
Liquefied carbolic acid	5 c.c.
Glycerine	50 c.c.
Distilled water	100 c.c.

The fuchsin and carbolic acid are thoroughly mixed in a glass dish, after which the glycerine and water are added. Before using, dilute four to ten times. This solution keeps well.

CZINZINSKI'S SOLUTION.

Concentrated aqueous solution of methylene-blue	50 c.c.
Eosin	0.5 gramme.
Absolute alcohol	70 c.c.
Distilled water	130 c.c.

NICOLLE'S CARBOL-THIONIN STAIN.

Saturated solution of thionin in 50 per cent.
 alcohol 10 c.c.
 1 per cent. aqueous solution of carbolic acid 100 c.c.

BOWHILL'S STAIN FOR FLAGELLA.

Solution (a): Saturated alcoholic solution of orcein (this solution possesses greater staining powers if allowed to stand ten days before use).

Solution (b): 20 per cent. solution of tannic acid in water, dissolved by heating.

The above solutions are mixed with distilled water before using, as follows:—

(a) 15 c.c. ; (b) 10 c.c. ; distilled water, 30 c.c. Mix and filter.

PITFIELD'S STAIN FOR FLAGELLA.

- (a) Cold saturated solution of alum 10 c.c.
 Saturated alcoholic solution of gentian-violet 2 c.c.

To this add :

- (b) Cold 10 per cent. aqueous solution of tannic acid 10 c.c.

MORDANTS.

GRAM'S IODINE SOLUTION.

Iodine crystals	1 gramme.
Iodide of potash	2 grammes.
Distilled water	300 c.c.

LÖFFLER'S MORDANT.

For the Demonstration of Flagella.

Dissolve by heating together : tannin, 2 grammes ; distilled water, 8 c.c.

To this solution add :

Concentrated cold watery solution of ferrous
 sulphate 5 c.c.

And

Concentrated alcoholic solution of fuchsin 1 c.c.

Shake well together, and after filtering, the mordant is ready for use.

BUNGE'S MORDANT.

- 1 part liquor ferri sesquichlor. diluted in 20 parts
 distilled water 25 c.c.
 Saturated aqueous solution of tannin 75 c.c.

N.B.—Recent researches show that it is unnecessary to add alkali or acid, as originally recommended by Löffler, or the hydrogen peroxide solution added by Bunge to his mordant. This mordant should be left standing exposed to the air for some days before being used.

CONTRAST-STAINS.

FRIEDLÄNDER'S PICO-CARMINE.

1. Dissolve 1 gramme of carmine in 50 c.c. of distilled water, and add 1 c.c. of liquor ammoniæ fortior.
2. To the above solution add a watery solution of picric acid until the resulting precipitate ceases to dissolve on shaking the mixture.

The further addition of a small quantity of ammonia dissolves the precipitate.

The addition of a few drops of carbolic acid prevents micro-organisms developing in the solution. Filter before using.

This stain keeps well.

WEIGERT'S PICRO-CARMINE.

1. Carmine 2 grammes.
Liquor ammoniæ fortior 4 c.c.

Mix and set aside for twenty-four hours.

2. To the above add concentrated watery solution of picric acid, 200 c.c.

Mix and set aside for twenty-four hours.

3. Add acetic acid with a dropper until a precipitate forms, then add ammonia in a similar manner until the solution again becomes clear.

The above solutions of picro-carmines are used as contrast-stains with the Weigert, Gram, and Claudius methods.

COCHINEAL-ALUM.

Cochineal (powdered)	10 grammes.
Ammonia-alum	10 grammes.
Water	1 litre.

Evaporate to one-half by heating, and when cold add a few drops of carbolic acid and filter.

BORAX-CARMINE.

Carmine	0.5 gramme.
Borax	2.0 grammes.
Distilled water	100 c.c.

Mix and boil. Stir well, and, at the same time, add a few drops of a 5 per cent. solution of acetic acid until the colour changes. Decant in twenty-four hours and filter, adding a few drops of carbolic acid to preserve the solution.

HÆMATIN-ALUM SOLUTION.

Solution (a)—

Hæmatin crystals	0.5 gramme.
Absolute alcohol	25.0 c.c.

Heat carefully in a water-bath until the hæmatin is dissolved.

Solution (b)—

Alum	25.0 c.c.
Distilled water	500 c.c.

Solution (a) is added to solution (b), and the mixture filtered. A crystal of thymol is then added to the filtrate.

METHODS OF CLEANING GLASSWARE.

COVER-GLASSES.

It is essential for bacteriological work that the cover-glasses should be clean. For this purpose newly purchased cover-glasses may be placed in a solution of nitric acid and water for twenty-four hours; removed, washed in water, and kept until required in alcohol. Though this procedure will suffice for ordinary purposes it is often necessary, especially when the cover-glasses are used for blood-films or flagella-staining, to proceed more rigorously. In the latter case one of the following methods may be used :—

Löffler's Method.

1. Warm the cover-glasses for some time in concentrated sulphuric acid.
2. Rinse in water.
3. Place until wanted in a mixture of equal parts of alcohol and ammonia.
4. Before use, dry with a cloth, from which all fat has been extracted.

Zettnow's Method.

1. Boil the cover-glasses for ten minutes in the following solution :—

Bichromate of potash	200 grammes.
Aqua fervida	2 litres.
Sulphuric acid (strong)	200 c.c.
2. Pour off this solution, and wash for five minutes in a weak solution of caustic soda.
3. Repeat processes 1 and 2; number 1 for only five minutes.
4. Wash with water, then with alcohol, and dry with a cloth from which all fat has been extracted.

METHOD OF CLEANING USED COVER-GLASSES.

1. Throw the used cover-glasses into a dish containing pure sulphuric acid, and leave them there for some days.
2. Pour off the acid, and wash with water.
3. Boil in a strong solution of caustic potash.
4. Wash in water.
5. Wash in equal parts of alcohol and ether.
6. Wash in benzine or xylol, and dry with a cloth from which all fat has been extracted.

METHOD OF CLEANING NEW TEST-TUBES, CULTURE DISHES,
FLASKS, ETC.

Method I. Clean new test-tubes before use with 1 to 2 per cent. solution of hydrochloric acid. Rinse thoroughly in running water.

Method II. 1. Take a strong glass rod and tie some cotton wool about one end with a piece of string.

2. Dip the cotton in strong nitric acid, and cleanse the inside of the tubes. Wash in three or four changes of water, and place them on the rack to drain.

3. Rinse with strong spirit, place on the rack to drain, and when dry they are ready for use.

METHOD OF CLEANING DIRTY OR USED TUBES.

1. Remove the cotton plugs, and steam the tubes in the sterilizer or autoclave for thirty minutes to dissolve media and disinfect the contents.

2. Pour out the contents, and place the tubes in a large enamel basin containing a weak solution of caustic soda or common washing soda.

3. Pour off the soda solution, and wash the tubes in three or four changes of water.

4. Clean the inside of each tube with weak nitric acid, using a cotton swab, or wash with an ordinary test-tube brush and Hudson's powdered soap.

5. Wash in several changes of water, rinse with spirit, and place on the rack to dry.

DIFFERENTIATING REAGENTS.

1. Distilled water.
2. Absolute alcohol.

3. Acid alcohol (90 per cent. alcohol 100 c.c., distilled water 200 c.c.), to which add the desired per cent. of acetic, hydrochloric, nitric, sulphuric, or oxalic acids.

4. Weigert's anilin oil, 2 parts ; xylol, 1 part.

5. Oil of cloves.

6. Gram and Claudius solutions (p. 54 and pp. 21, 22).

These reagents act as follows : Distilled water removes surplus stain. Absolute alcohol dehydrates and removes stain. Acid alcohols are used to decolorize cover-glass specimens and sections, in order to differentiate micro-organisms which do not, in some instances, decolorize as readily as the tissues. Anilin oil and xylol are recommended by Weigert in his stain for fibrin or bacteria in sections. Oil of cloves is used for sections in the Claudius method.

CLEARING AND MOUNTING MEDIA.

The clearing media commonly used are : xylol, oil of cedar, turpentine, oil of origanum, oil of Bergamot. Temporary mounts of cover-glass specimens may be made with cedar oil. The best and most usually employed mounting medium is Canada balsam, which has been rendered suitably fluid through the addition of xylol. For certain purposes glycerine and gelatine may be used (gelatine 10 grammes, glycerine 40 c.c., water 20 c.c. Place in a water-bath and heat until the gelatine is dissolved.)

DISINFECTING SOLUTION FOR POTATOES, HANDS, ETC.

Bichloride of mercury	.	.	1 gramme.
Water	.	.	1000 c.c.
Hydrochloric acid (strong)	.	.	5 c.c.

PART II.

The Preparation of Nutrient Media.

ORDINARY BOUILLON.

1. CUT up 500 grammes of lean beef or veal, freed from fat, into small pieces, add 1 litre of distilled water, and set aside in a cool place for twelve to twenty-four hours. In hot weather place in an ice-chest, or boil forthwith over an open flame for one hour, stirring continually.

2. Place the mixture in a clean piece of cloth or fine muslin, strain into a flask, and squeeze until you obtain 1 litre of meat infusion.* It has an acid reaction.

3. Add, Dry peptone . . 10 grammes.
 Common salt . . 5 grammes.

4. Heat the flask in the water-bath for one to one and a half hours, or warm directly over the flame, using an ordinary enamel sauce-pan with several pieces of wire gauze or a sheet of asbestos placed between the flame and the pan.

5. Render *slightly* alkaline by means of a saturated solution of sodium carbonate—if *too much alkali is added the bouillon will never clarify*. The white of a hen's egg can be added, but it is not absolutely necessary.

6. Sterilize in the steam sterilizer one to two hours; remove, and when *cold*, filter through two thicknesses of filter-paper. If filtered when hot, fatty substances pass through. *Filter very slowly*.

7. Place again in the steam sterilizer for one hour. On removal the bouillon should be of a clear golden colour. If it is *turbid* it is

* Instead of using lean beef or veal to make a meat-infusion, Liebig's Meat-Extract can be substituted, using 10 grammes of the extract to 1 litre of distilled water. The resulting medium has a brownish colour, whereas with the meat-infusion it is almost colourless.

allowed to cool, and is then again filtered, sterilized for one hour, and the process repeated until it is thoroughly clarified.

8. Pour the bouillon into clean tubes (about 10 c.c. in each), or else into small Erlenmeyer flasks, and close with cotton plugs. (*In making a cotton plug, twist the cotton together, and when placed in the tube the relaxation of the twist ensures a tight fit.*)

9. The tubes or flasks are finally sterilized in the steam sterilizer for fifteen to thirty minutes on each of three successive days. The bouillon is ready for use if on cooling it still remains perfectly clear.

GRAPE- OR MILK-SUGAR BOUILLON.

Put half a gramme of grape-sugar or 2 grammes of milk-sugar into a small Erlenmeyer flask. Add 100 c.c. of bouillon after it is filtered, and proceed as above, beginning at 7.

GLYCERINE BOUILLON.

Glycerine bouillon is prepared by adding 4 to 6 grammes of glycerine to each 100 c.c. of the ordinary bouillon described above, the glycerine being added at stage 3 or 7 in the preparation of the plain bouillon.

CARBOL BOUILLON.

Carbol bouillon is prepared by adding 6 c.c. of a 5 per cent. watery solution of pure carbolic acid to every 100 c.c. of ordinary bouillon. The amount of carbolic acid will naturally vary according to requirements.

POTATO MEDIA.

ORDINARY METHOD.

1. Select some good potatoes and wash them thoroughly with the potato-brush and water, cutting out any eyes with the potato-knife.

2. Lay the cleansed potatoes in sublimate solution (p. 58), for thirty minutes.

3. Sterilize in the steam sterilizer one-half to three-quarters of an hour.

4. Wash the hands in sublimate solution, remove the potatoes, and cut in two with a sterilized potato-knife.

5. Lay the cut pieces of potato in deep culture dishes with a piece

of filter-paper previously soaked in the sublimate solution in the bottom of the dish. The potatoes must be quite cooled before being inoculated.

ESMARCH'S METHOD.

1. Thoroughly wash some potatoes.
2. Peel and cut off the ends.
3. Cut into sections 1 cm. thick and place in clean water.
4. Place the slices in Esmarch dishes.
5. Sterilize in the steam sterilizer three-quarters of an hour.
6. If the Esmarch dishes are not sterilized before the slices of potato are put in, then sterilize in the steam sterilizer on three successive days. On the first day, for thirty minutes; on the second and third days, for fifteen to twenty minutes.

ROUX AND GLOBIG'S METHOD.

1. The potatoes are thoroughly washed, the ends cut off, bored through with a cork borer, and the cylindrical pieces of potato laid in water.
2. The potato-cylinders are cut in two diagonally and placed again in water.
3. Take some ordinary test-tubes, place some cotton or a piece of glass rod in the bottom, add a little water, introduce a piece of potato, and shake it down until it touches the support in the bottom of the tube. Plug the tube with cotton.
4. Potatoes as a rule have a slightly acid reaction. To render them faintly alkaline, lay the potatoes in 1 per cent. solution of sodium carbonate before placing in the tubes or before sterilizing.
5. Sterilize on each of three successive days as described in preceding method.

HOLZ'S POTATO-WATER GELATINE FOR CULTIVATION OF
TYPHOID BACILLI.

1. Wash and peel 500 grammes of potatoes; grate very fine, and squeeze the juice through a linen cloth.
2. The juice is left twenty-four hours in an ice-chest before filtering, or is filtered immediately through animal charcoal.
3. Sterilize one hour in the steam sterilizer; add 10 per cent. of gelatine (Elsner adds 1 per cent. of iodide of potash to the gelatine

before use) ; sterilize again one hour in the steam sterilizer, and fill into test-tubes.

4. Sterilize the tubes on each of three successive days as in von Esmarch's method, described above.

POTATO-WATER FOR CULTIVATION OF BACILLUS TUBERCULOSIS.

1. Grate 500 grammes of clean peeled potatoes ; add 500 c.c. of water, and place in an ice-chest for twelve to twenty-four hours.

2. Decant the liquid portion, and add enough distilled water to make 1000 c.c.

3. Boil one hour in the water-bath ; filter ; add 4 per cent. of glycerine, and fill into tubes.

4. Sterilize the tubes on each of three successive days as in von Esmarch's method, described above.

PEPTONE MEDIA.

DUNHAM'S PEPTONE SOLUTION.

Dried peptone (Witte)	.	.	1 grammé.
Sodium chloride	.	.	0.5 gramme.
Distilled water	.	.	100 c.c.

Boil until the peptone is dissolved. If it is neutral or slightly alkaline it is not necessary to add alkali. Filter, place in tubes, and sterilize by the ordinary discontinuous method on three successive days.

This medium is useful for the cultivation of cholera vibrios and the like, and is used for the cultivation of organisms when it is desired to test their power of producing indol.

PEPTONE ROSOLIC ACID SOLUTION.

Dunham's peptone solution . 100 c.c.

Add 2 c.c. of the following solution :—

Rosolic acid (Coralline)	.	.	0.5 grammé.
Alcohol (80 per cent.)	.	.	100 c.c.

Boil, filter, place in test-tubes, and sterilize by the ordinary discontinuous method on three successive days. This medium is used to study the reactions (acid or alkaline) produced by different bacteria.

MILK MEDIA.

PLAIN MILK.

The milk must be quite fresh and the reaction must not be acid. Place about 10 c.c. in each tube, plug, and sterilize in steam sterilizer for one hour on each of three successive days. When the tubes are removed from the sterilizer they should be kept at about 20° C.

The proper sterilization of milk media is very important, as the spores of some of the bacteria which occur in milk are most resistant.

LITMUS MILK.

This medium is prepared by adding tincture of litmus to the milk (until it is slightly blue in colour) before sterilization. The litmus serves as a useful index of alterations in reaction. Ordinary milk acquires a light brown colour after sterilization.

FREUDENREICH'S METHOD OF PREPARING SOLID MILK MEDIA.

Take a tube containing 5 c.c. of sterilized milk, and mix the milk with 5 c.c. of sterile, 20 per cent. gelatine, or 2 to 3 per cent. agar in a sterile Petri-dish. This medium is not transparent.

MILK-PEPTONE GELATINE.

1. Heat 1 litre of milk to 60° to 70° C. in an enamel pot.
2. Add 60 to 100 grammes of gelatine, and heat until the casein is coagulated.
3. Place the mixture in a thin linen cloth and squeeze until the fluid portion is separated from the casein.
4. Place the still warm fluid portion in the incubator to allow the fat to come to the surface, then cool, and remove the fat.
5. Heat again to the boiling-point, and add—

Peptone (Witte's)	.	.	10 grammes.
Common salt	.	.	5 grammes.

6. Neutralize or make slightly alkaline, and filter, using a hot-water funnel.

The first portion that comes through the filter (about 15 to 20 c.c.) is usually cloudy, and is thrown away. The remainder is usually clear, either slightly or not at all coloured, and does not become cloudy on further heating and cooling. *Milk-Peptide Agar* is prepared in a similar manner, but is cooked for a considerably longer time.

EGG MEDIA.

HUEPPE'S METHOD.

1. Wash a fresh egg with a brush, soap and water, and sublimate.
2. Sterilize a covered glass dish with sublimate solution, wash with sterilized water, and dry with sterilized wadding.
3. Make an opening at one end of the egg with a hot needle and inoculate the egg.
4. Close the opening in the egg-shell with a small piece of tissue paper, cover it with a coating of collodion, and place it in the dish.

GÜNTHER'S METHOD.

1. Wash one end of an egg with a brush, soap, and water.
2. Sterilize the end of the egg with the flat surface of a clean hot potato-knife.
3. Heat a steel needle, and when cool, make an opening in the sterilized spot, large enough to admit the platinum needle with which the egg is inoculated.
4. Close with sterile paper and collodion, or with hot sealing-wax.

EGG ALBUMEN.

Take the white of an egg and distribute it in tubes, slant, and coagulate as with blood-serum (see p. 68).

GELATINE MEDIA.

NUTRIENT GELATINE.

1. Make 1 litre of meat-infusion (see under "Bouillon," 1 and 2, p. 59).

2.	Gelatine	.	.	.	100 grammes.
	Dry peptone.	.	.	.	10 "
	Common salt*	.	.	.	5 "

are placed in a glass flask or sauce-pan, and the meat-infusion added last.

3. The gelatine and peptone are thoroughly dissolved in a water-bath, at 40° to 50° C., or directly over the flame, using an ordinary enamel sauce-pan, with several pieces of wire gauze or a sheet of asbestos intervening between the flame and the pan.

* For the cultivation of marine bacteria, 3 per cent. NaCl is added.

4. Test the reaction, rendering it alkaline with a concentrated solution of sodium carbonate (the same precaution is necessary as described under "Bouillon," 5, p. 59), and add a fresh egg.

5. Place in the steam sterilizer for one and a quarter hours (too long boiling lowers the solidifying-power of the gelatine), remove and filter through *two thicknesses* of filter-paper. Fold the filter-paper with a sharp point pushed well down into the neck of the glass funnel, which is placed obliquely in the flask, a small Bunsen flame being fixed at an angle a short distance from the neck of the glass funnel. The gelatine filters easily by this method, and a hot-water funnel is unnecessary.

6. The reaction is again tested, and if still faintly alkaline, fill about 10 c.c. into each tube and close with cotton plugs. The old method of sterilizing wadding and tubes before use is now frequently dispensed with, except for blood-serum.

7. Sterilize the tubes of gelatine in the steam sterilizer for fifteen minutes on each of three successive days. The gelatine should finally be as clear as glass.

GRAPE- OR MILK-SUGAR GELATINE.

To 100 c.c. of nutrient gelatine, before it is sterilized (see above), add (at stage 6) 2 grammes of grape- or milk-sugar.

CARBOLIC ACID GELATINE.

To every 100 c.c. of liquid 10 per cent. gelatine, add 4 c.c. of a 5 per cent. watery solution of pure carbolic acid.

WORT GELATINE.

1. Take 1 litre of wort. Add 100 grammes of gelatine, and heat in the water-bath until the gelatine is melted.

2. Place in the steam sterilizer for thirty minutes.

3. Filter and fill into tubes or Erlenmeyer flasks.

4. Sterilize in the steam sterilizer for twenty minutes on each of three successive days.

AGAR-AGAR MEDIA.

ORDINARY AGAR.

1. Prepare 1 litre of meat-infusion (see "Bouillon," 1 and 2, p. 59), and add

1 per cent. of dry peptone	10 grammes.
$\frac{1}{2}$ per cent. of common salt	5 grammes.

2. Place the above in a clean flask; heat in a water-bath one to one and a half hours, and add 1 to 2 per cent. ($1\frac{1}{2}$ is the amount generally used) of agar, finely powdered, or cut into very small pieces. Boil five to eight hours until the agar is thoroughly dissolved.

3. Render alkaline with sodium carbonate solution.

4. Place in the steam sterilizer one to two hours.

5. Filter through ordinary filter-paper or flannel, keeping it in the steam sterilizer.*

6. Fill the desired quantity into test-tubes, and close with cotton plugs.

7. Sterilize in the steam sterilizer for fifteen minutes on each of three successive days.

A RAPID METHOD OF PREPARING AGAR.

1. Cut up 500 grammes of lean beef or veal (freed from fat) into small pieces, and place in an enamel sauce-pan. Add 1 litre of water, and mix thoroughly.

2. Heat the mixture over a sand-bath (gently at first), and, after it has cooked for one and a half hours, filter through ordinary filter-paper.

3. Add 15 grammes of agar, cut into very small pieces, and cook over the sand-bath for one hour; then add

Peptone (Witte's)	10 grammes.
Common salt	5 grammes.

When these are dissolved, neutralize with saturated solution of sodium carbonate until a decided but not too intense alkaline reaction is obtained.

4. When the mixture has cooled to 50° C. add the whites of two fresh eggs, and shake the mixture very thoroughly. Successful filtering depends on the mixture being thorough. For this purpose

* The author has had good results from filtering agar under pressure through two pieces of moleskin cloth.

the author has found it convenient to place the mixture in a large white porcelain bowl, thorough mixture being secured by means of a "Dover" egg-beater.

5. The thoroughly shaken mixture is again placed on the sand-bath and heated until the temperature of the sand close to the enamelled vessel registers over 100° C. (at a lower temperature filtration is imperfect).

6. Cook one and a half to two hours, and then filter through an ordinary folded filter-paper previously moistened with boiling water. If properly prepared the agar will filter as quickly as ordinary bouillon, no hot water funnels, etc., being necessary. The whole process is completed within six hours.

GRAPE-SUGAR AGAR.

Add half a gramme of grape-sugar to 100 c.c. of ordinary agar after it is filtered, and proceed as above at stage 6. In filtering grape-sugar agar into tubes it is customary to fill the tubes two-thirds full, as this medium is usually employed in the cultivation of anaërobic bacteria.

GLYCERINE AGAR.

Add 4 to 6 grammes of glycerine to 100 c.c. of ordinary agar, either before or after filtering, and proceed as above with ordinary agar, from stage 6.

WURTZ'S LACTOSE-LITMUS AGAR.

1. To ordinary slightly alkaline agar add 2 to 3 per cent. of lactose.

2. Fill into tubes and sterilize by the ordinary method on three consecutive days.

3. When sterilized, add enough *sterilized litmus* tincture to give the medium a decided pale-blue colour.

Bacteria causing fermentation of lactose, when grown in this medium, develop colonies of a pale-pink colour with a corresponding reddening of the surrounding medium. Bacteria incapable of producing fermentation form pale-blue colonies and cause no reddening of the surrounding media. (Instead of agar, ordinary nutrient gelatine can be substituted in the preparation of this medium.)

BLOOD AGAR.

1. A few drops of human, pigeon's, or other blood obtained under

aseptic precautions, are spread over the oblique surface of ordinary sterile agar-agar tubes, or upon agar solidified in Petri-dishes.

2. The prepared tubes are placed in the incubator at 37° C.; the tubes which become contaminated are discarded. (This medium is specially adapted for the gonococcus and influenza bacillus.)

AGAR GELATINE.

This medium is prepared in the same way as ordinary agar (see above), using 1.5 grammes of agar, and when it is dissolved adding 50 grammes of gelatine.

BLOOD-SERUM MEDIA.

FLUID BLOOD-SERUM.

1. Collect the blood from a living animal into a large sterile glass jar, under aseptic precautions, and close the jar tightly.
2. Place the jar in an ice-chest for twenty-four hours.
3. Remove the serum into tubes with a sterile pipette.
4. Place the tubes of serum in the incubator at 37° C.
5. Remove any tubes that become turbid.

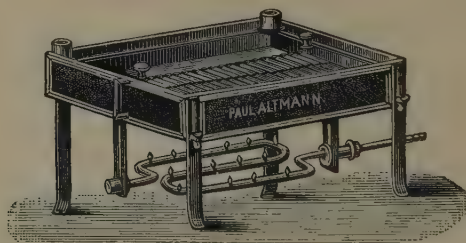


FIG. 10.—Koch's Apparatus for Solidifying Blood-Serum.

SOLID BLOOD-SERUM (GELATINIZED).

1. Collect the blood in the same manner as above.
2. About fifteen minutes after it has begun to clot, pass a sterilized glass rod between the clot and the wall of the jar, breaking up the adhesions that prevent the clot from sinking.
3. Close the jar tightly, and place in an ice-chest twenty-four to forty-eight hours.
4. Draw off the serum with sterilized pipettes and transfer to

culture-tubes. (*This is the only medium where it is essential for the tubes and plugs to be sterilized before filling.*)

5. Place the slanted tubes in Koch's blood-serum sterilizer (see Fig. 10), and heat to 65° or 68° C. for one hour on each of five successive days, and place in the incubator at 37° C. for several days to test whether they are sterile.

6. Place the sterile tubes in Koch's apparatus for solidifying blood-serum, and heat to 75° or 80° C. until the serum is solidified.

SOLID BLOOD-SERUM (COAGULATED).

This medium is prepared as described in the preceding paragraph up to stage 4.

5. Place the tubes in Koch's serum sterilizer and heat at once to 90° or 95° C. for one hour or more.

6. Place the tubes in an inclined position in the steam sterilizer for one hour or more. The temperature must not rise too high, otherwise bubbles form in the serum.

7. Place the tubes in the ordinary wire baskets, and sterilize as usual for fifteen minutes on each of three successive days.

Serum prepared according to this method is more opaque than when prepared according to the preceding method, but it answers all practical purposes, saves time, and avoids the chance of imperfect sterilization, which is somewhat common when using the gelatinizing process.

SPECIAL MEDIA.

LÖFFLER'S METHOD FOR CULTIVATING THE BACILLUS DIPHTHERIÆ.

1. Take blood-serum, 3 parts; 1 per cent. grape-sugar bouillon, 1 part. Mix thoroughly, and fill into test-tubes.

2. Solidify at between 60° and 70° C.

3. Sterilize for ten minutes in the steam sterilizer on each of three consecutive days.

NUTTALL'S METHOD OF SECURING STERILE FLUID SERUM.

Nuttall has devised a bulb for the collection of blood-serum. It is a pear-shaped sterilizable vessel made of glass, in which 10 to 100 c.c. of blood can be collected. Under proper precautions no contamination takes place. The bulb is provided above with a neck (which is closed with cotton), and below with a tubule which projects at almost a right angle to the long axis of the bulb. The bulbs are readily made out

of one piece of tubing by any moderately expert glass-blower. The method of procedure is as follows :—

Expose the femoral or carotid artery, and adjust two ligatures ; the one distant from the heart is tightened and the proximal one left loose between the latter and the heart. The artery is clamped, a small slit is now made in the wall of the vessel, the point of the tubule, the sealed end of which has been broken off and rounded in the flame, is introduced and the artery bound tightly around it with the loose ligature. The clamp is then removed, when the bulb is quickly filled with blood. Replace the clamp, remove the bulb, and seal in the gas flame. The loose ligature is now tightened and the wound closed. The glass bulb is put in a cool place until coagulation has occurred. The serum is withdrawn with a sterile pipette.

FOULERTON'S MEDIUM FOR CULTIVATING THE GONOCOCCUS.*

Mix 2 parts of ordinary agar with 1 part of human urine containing 5 per cent. of egg albumen. Fill into tubes and sterilize by the usual discontinuous method.

MEDIUM FOR MOULD FUNGI.

1. Fill some finely grated dry bread into Erlenmeyer flasks or test-tubes in a layer about $1\frac{1}{2}$ inches high.
2. Add enough water to convert the bread into a paste, and close the tubes or flasks with plugs.
3. Sterilize in the steam sterilizer for fifteen minutes on each of three successive days.

The bread-paste having an acid reaction is unsuitable for the cultivation of most bacteria.

SABOURAUD'S MEDIUM FOR THE FUNGUS OF FAVUS, ETC.

Peptone	.	.	.	0.8 gramme.
Mannite	3.8 grammes.
Distilled water	.	.	.	100 c.c.
Agar	.	.	.	1.4 gramme.

Prepare in the ordinary manner, render slightly alkaline, fill into tubes, and sterilize fifteen minutes on each of three successive days.

* *Transactions of the British Institute of Preventive Medicine*, vol. i., 1897.

MEDIUM FOR NITRIFYING BACTERIA.

WINOGRADSKY'S SOLUTION.

Ammonium sulphate	.	.	1 gramme.
Potassium phosphate	.	.	1 gramme.
Pure water	.	.	1000 c.c.

Pour 100 c.c. of the above solution into each flask, and add to each 0.5 to 1.0 gramme of basic magnesium carbonate, suspended in distilled water, and sterilize by boiling.

FILLING TUBES OR FLASKS WITH MEDIA.

Fill the desired amount of medium into the tubes from a small Erlenmeyer flask, or use a funnel with a pinch cock prepared for the purpose when exactitude is necessary, but for all practical purposes an Erlenmeyer flask is sufficient. Care must be taken that none of the material is dropped against the inside of the mouth of the test-tube, as it will cause the plugs to adhere. The filled tubes are plugged, and the tubes sterilized in the steam sterilizer for fifteen to twenty minutes on each of three successive days.

INOCULATION OF MEDIA.

1. The platinum wires or loops should always be sterilized in the flame of a Bunsen burner, both before and after use. In doing this the wire should be held point downwards, almost perpendicularly, in the flame, the adjacent portion of the glass or metal rod it is attached to being passed several times through the flame.

2. Fluid cultures are inoculated by means of the sterilized platinum loop.

3. Gelatine and agar stab-cultures are made with the platinum needle, usually only one stab being made in each tube, the stab penetrating almost to the bottom of the medium.

4. Agar-gelatine and potato surface-cultures are made with the platinum loop, the material being spread over the surface of the medium.

METHOD OF MAKING INOCULATIONS OF MEDIA FROM HARD SUBSTANCES,
FAVUS CRUSTS, ETC.

1. Sterilize a large watch-glass in the flame and cover it up.

2. Sterilize the end of a clean glass rod in the flame. After allowing the glass to cool :

3. Place a small quantity of sterilized bouillon in the watch-glass, add the material to be investigated, triturate with the glass rod until thoroughly reduced and mixed with the bouillon, and proceed as in previous method (stage 2).

CULTURE METHODS.

KOCH'S ORIGINAL METHOD FOR THE ISOLATION OF GERMS IN PURE (PLATE) CULTURE.

1. Take three tubes containing about 10 c.c. of sterilized nutrient gelatine, and melt the medium by heating in the water-bath at 40° C.

2. When the tubes have cooled to 30° C., remove the plug from the first tube, heat the mouth of the tube in the Bunsen flame, inoculate the medium with a platinum loop of a liquid culture, or a trace of any other desired material. After inoculation distribute the organisms evenly, allowing the fluid to flow gently backward and forward, not allowing it to come in contact with the plug. Too much agitation

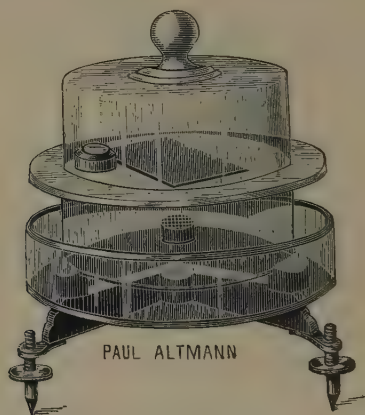


FIG. 11.—Koch's Plate-Culture Levelling Apparatus.

should be avoided as this causes air-bubbles to form. The tube is held between the thumb and first finger of the left hand, while the plug is placed between the first joints of the first and second fingers of the same hand. This is now known as the original tube, and marked "O."

3. Take another tube of gelatine, remove the plug, placing it between the second and third fingers of the left hand, sterilize the mouth of the tube, place it alongside the original tube, and with a sterilized platinum loop transfer three loops of the gelatine in the original tube into the second tube. Replace the plug in the original

tube and set it on one side. The second tube is known as No. 1 dilution, and marked "1."

4. The third tube of gelatine is similarly inoculated with three loops from the second tube (No. 1 dilution) in the same manner, and is known as No. 2 dilution, and marked "2." (For marking the tubes for future identification, yellow or blue pencils for writing on porcelain, metal, or glass, will be found very convenient.)

5. Three sterilized glass plates are now removed from the plate-box, placed one above the other on the horizontal ground glass plate of the plate-culture apparatus (see Fig. 11), and the contents of the above tubes poured in order on the plates. When the gelatine is thoroughly set, the plates are placed on glass benches one above the other, the first plate ("O") being at the bottom, in a plate-culture dish.

PETRI-DISH CULTURES.

Instead of using plates, it is simpler to pour the gelatine into sterilized Petri-dishes. The prepared plates or Petri-dishes can be placed in the incubator at 22° C., or left to develop at room temperature, as a rule only two of the plates are fit for future observation—the original plate generally containing too many colonies. When finished, sterilize the platinum wires, and place the empty tubes in the disinfecting solution.

AGAR PLATES.

In making agar-agar plate-cultures the fact must not be lost sight of that this medium solidifies at 39° C.

1. Melt three tubes of agar at 90° C. (*freshly prepared tubes must not be used, as the water of condensation will cause the surface colonies to run together*).

2. Place in a water-bath at 40° C., remove, and inoculate quickly, making the necessary series, O – 1 – 2.

3. Pour the contents of the tubes preferably into Petri-dishes, or if plates are used, place a spot of melted sealing-wax at each corner to prevent the agar-agar sliding off the plates. To prevent sliding off of the agar, keep the plates perfectly horizontal whilst transferring them to the dish after they have been hardened on the levelling apparatus.

METHOD WITH ORDINARY AGAR IN TUBES.

1. Take three freshly-prepared tubes of obliquely solidified agar,

—usually called “agar-slants”—with plenty of water of condensation in the bottom.

2. Inoculate only the water of condensation and transfer the bacterial suspension to the water contained in the series of tubes, making the usual number of dilutions, O – I – 2.

3. When the dilutions are completed, allow the water of condensation in each tube to flow gently over the oblique surface of the agar medium, and place in the incubator at 37° C.

BLOOD-SERUM AND AGAR PLATE METHOD.

1. Take three tubes of sterilized agar, melt, maintain the agar at 40° C. in a water-bath, inoculate, and make the usual dilutions, O – I – 2.

2. Pour the contents of the tubes into Petri-dishes, and add sterilized liquid blood-serum heated to 40° C., mixing it thoroughly with the agar. (This method is specially adapted for the growth of diphtheria bacilli and gonococci.)

AGAR STROKE-CULTURE PLATE METHOD.

1. Liquefy some agar tubes, pour the medium into Petri-dishes, and allow it to solidify.

2. Dip a sterilized platinum wire in the suspected material, raise the lid of the Petri-dish obliquely, and make several strokes with the wire across the surface of the agar. Or, instead of using dishes, take six to eight agar slants and inoculate the series by making strokes on the surface of the medium, not sterilizing the needle until the last tube of the series has been inoculated.

ROLL-CULTURES.

The original von Esmarch Method.

1. Take three large wide-mouthed tubes containing sterilized nutrient gelatine.

2. Liquefy the gelatine, and inoculate it with the material under investigation, making the usual dilutions, O – I – 2.

3. Push the cotton plugs well down, cut off the protruding ends, and put on india-rubber caps.

4. Cool the gelatine by twisting the tube round upon its longitudinal axis in ice-water, when the gelatine will adhere to the

inner walls of the tube in a thin film. Before putting the tube in the ice-water, roll the gelatine round the periphery of the internal portion of the cotton plug ; by this means the centre of the plug remains free from gelatine, otherwise an air-tight cavity results.

5. To remove a colony from a roll-culture, place the tube under a low power lens (see special apparatus, Fig. 14), select the colony, mark the outside of the tube over the colony, and then remove to other media, etc., with a bent platinum wire, the spot outside acting as a guide.

Booker's Method.

A much better method than the preceding, though depending upon the same principle, is that recommended by Booker.

1. Place a block of ice of convenient size in a dish, resting it upon a towel (which prevents it slipping).

2. Take a plugged test-tube filled with warm water, lay it upon the block and thereby melt a groove in the ice.

3. The test-tubes (best filled with 5 to 6 c.c. of medium) are placed in the groove after inoculation, and revolved rapidly with the fingers of the right hand. The left hand holding the dish regulates the obliquity of the tube, which at the beginning of the rotation should have the cotton plug at a higher level. The medium should not come in contact with the plug.

This method can be used also for agar, but the tubes must be kept slanted for about twenty-four hours, otherwise the agar will not keep its position in the tube. After a certain number of hours the agar in the vicinity of the cotton plugs dries and adheres to the glass ; with gelatine no such difficulty occurs. Rubber caps are not necessary.

Nuttall's Method.

The Booker method requires that one shall have a piece of ice of suitable size at hand, and the continued melting of the ice alters the shape of the groove. Whereas the Booker method will always be useful where ice is readily obtained, especially in summer, a useful substitute for the ice-block will be found in the apparatus about to be described. The apparatus is always at hand (ice is not), and the cold water, as it runs from the tap, suffices during the greater part of the year for chilling the tubes. In summer, ice-water can be allowed to run

upon the tubes from a reservoir overhead. In this apparatus the form of the groove remains constant.

"The apparatus consists of two parts: (1) A block of marble; (2) a metal receptacle with attachments. The block ($21 \times 17 \times 5$ cm.) is provided upon its upper polished surface with two grooves (*b*) of a size adapted to tubes of different sizes (the one groove is 17, the other 13.5 cm. long). In cross section, the grooves must represent somewhat less than a half circle, otherwise the water which accumulates between the surfaces of the tube and groove exerts such an amount of capillary attraction as to seriously interfere with the rotation of the tube. On the right hand side of the block, about 1 cm. from its edge, is a single groove deeper than the preceding which it traverses. This groove serves to drain off the water which would otherwise run along the length of the tube and wet the plug. Owing to the capillary attraction before mentioned the tubes will not rotate properly within a polished marble groove. They, however, rotate very rapidly when the surface of the groove is covered by a thin coat of

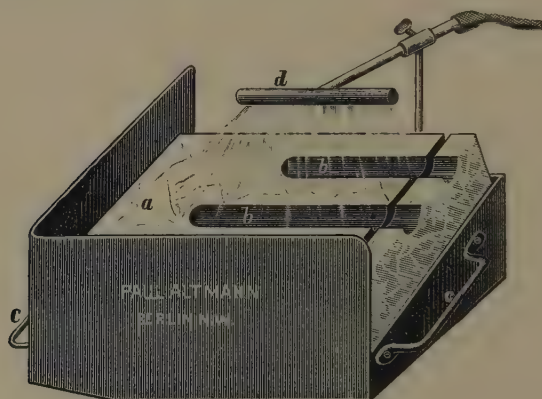


FIG. 12.—Nuttall's Roll-Culture Apparatus.

paraffin, which is applied in a liquefied condition by means of a brush and smoothed with a heated glass tube. The layer of paraffin requires to be occasionally renewed, the dried block being previously wiped off with a cloth moistened with xylol or turpentine. As seen in the figure, the block is held in a slanting position within the receptacle, which is made somewhat larger than the block, so as to catch any water which may flow over its sides. The shape of the receptacle is adapted to that of the block, the front and left side being high so as to catch the water as it splashes from the rotating tube. At the back is attached a verticle rod, with a screw attachment to hold a perforated brass T-tube (*d*) through the four openings of which water is allowed to fall upon the tubes. The floor of the receptacle is flat. On the left side is a tube to which a piece of rubber tubing is attached, the same serving to drain off the water which flows into the receptacle. The receptacle rests upon two sleigh-like cutters which are cut away somewhat at the left side, so that by pressing down upon the left handle (*c*) of the receptacle it is possible to give the whole

apparatus a decided tilt to one side. As soon as this handle is released, the apparatus returns by its own weight to the horizontal position. On the right of the receptacle is a second handle, which, together with the first, serves for carrying the apparatus about. These handles should be made sufficiently wide to admit the four fingers of each hand.

"When the apparatus is to be used, water is allowed to flow with *moderate* rapidity from the spray, the left handle is pressed down and the culture tube placed in the groove, rotation beginning immediately. As soon as the tube is rotating properly, the apparatus is allowed to gradually resume the horizontal position, and the rotation is continued until the medium has solidified. Solidification occurs rapidly with agar, more slowly with gelatine, so that when the latter is used, it will save labour (when the water is not especially cold), to bring down its temperature to near the point of solidification before placing it in the apparatus. The tubes should be rotated only in one direction (towards the operator), using the tips of one or two fingers gently. Splashing can be considerably reduced by moving the finger backward and forward *between* the small water jets (through a mistake of the draughtsman the T-tube is made to appear as if the water came out in numerous jets). By placing the apparatus in a slanting position it can also be used for slanting media." (Herr Paul Altmann, Luisenstrasse 52, Berlin, N.W., will supply a thoroughly well-made apparatus complete for 25 marks.) *

Gelatine plate, dish or roll cultures, are kept at ordinary room temperature or in the incubator at 22° C., and agar ones at 37°. In twenty-four to seventy-two hours they can be examined with the naked eye, hand-lens, or microscopically with a low power objective.

METHODS OF COUNTING THE COLONIES WHICH DEVELOP IN CULTURES ON SOLID MEDIA.

When the original plate-culture method of Koch is used, the

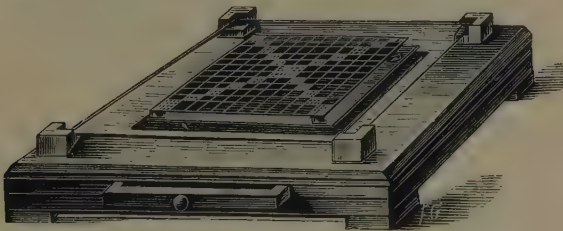


FIG. 13.—Wolffhügel's Counting Apparatus.

number of colonies that have developed in the plate is determined by means of Wolffhügel's counting apparatus (Fig. 13). This consists of a plate of glass upon which squares have been ruled by means of a diamond. The ruled plate is attached to a wooden support by hinges,

* *Philadelphia Med. Journ.*, 1900.

so that it can be placed at an angle or horizontally as required. The plate culture is laid upon the ruled plate, and the number of colonies estimated or counted by counting the colonies which lie within a series of squares.

When Petri-dishes are used, this apparatus can be also employed, the area of the plate being duly calculated. More convenient are ruled discs of glass or paper, various patterns of which can be obtained from dealers in bacteriological apparatus at small cost. The principle in both cases is the same. Where the colonies are large and numerically small, they may be counted with the naked eye, whereas when they are numerous and small, a magnifying-glass of low power is used. To be useful for counting colonies the microscope should be provided with a large stage.

For counting colonies in roll-cultures, an apparatus designed by

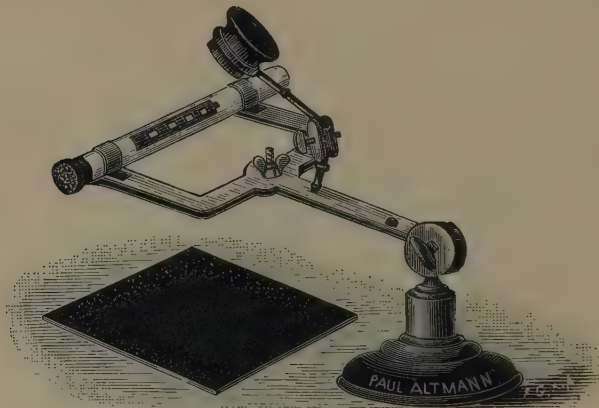


FIG. 14.—Esmarch's Apparatus for Counting Roll-Culture Colonies.

von Esmarch (see Fig. 14), may be used. It consists of a test-tube holder, and lens, attached to a stand. The holder contains quadrangular apertures of different sizes, the number of colonies being counted in the larger or smaller apertures depending on the number of colonies in the culture. A simpler method consists in attaching a piece of paper, in which squares have been cut, to the tube by means of elastic bands, and using an ordinary hand or watchmaker's lens (Nuttall). The estimation of the total number is made, as with plate-cultures, by measuring the surface of the glass covered by the media, and multiplying by the number of colonies found in a given square.

QUANTITATIVE PLATE-CULTURE METHOD.

This method is used to determine the number of bacteria in a given quantity of material. Fluids are examined as follows :—

1. Transfer with a sterilized capillary pipette known quantities of dilutions, say, 0.01 to 1 c.c. of the material into a series of tubes containing 10 c.c. of liquefied gelatine.
2. Pour the contents of the tubes on plates or into Petri-dishes, or make roll-cultures, and set aside for from twenty-four hours to seven days.

When the colonies have developed, the exact number is ascertained by the methods above described.

METHOD OF OBTAINING A PURE CULTURE FROM A COLONY ON A PLATE OR DISH.

1. Examine the plate under the microscope with a low power lens. Select an isolated colony of the desired organism.
2. Remove a part of the colony on the point of the sterilized platinum wire.
3. Remove the plug from a gelatine or agar tube, holding the tube perpendicularly, mouth downwards, in the left hand, and make a stab with the needle extending almost to the bottom of the tube, in the middle of the medium. Heat the neck of the tube in the flame, and replace the plug ; with liquid media the tube is held slanting, mouth upwards.

MAINTAINING PURE CULTURES IN THE LABORATORY.

1. Inoculate the cultures into fresh medium every two or three weeks.
2. Place the tube containing the original culture between the *thumb* and *first* finger of the *left* hand, and place the tube to be inoculated between the *first* and *second* fingers of the same hand.
3. Remove both cotton plugs, and hold between third and fourth and fourth and fifth fingers respectively of the left hand.
4. Pass a sterilized platinum needle or loop into the original tube without touching the sides, and transfer the material into the second tube, using similar precautions.
5. Heat the necks of both tubes in the flame, and scorch both plugs before returning them.

METHODS OF CULTIVATING ANAEROBIC BACTERIA.

Anaerobic organisms are characterised by their inability to grow in the presence of oxygen, and many devices are employed for the exclusion of oxygen from the cultures.

The preparation of suitable media and cultivation of anaerobic bacteria require skill and knowledge of bacteriological technique.

KOCH'S METHOD.

Inoculate a gelatine plate, and cover the gelatine with a thin piece of sterilized mica.

HESSE'S METHOD.

Pour some sterilized oil on the surface of a gelatine stab-culture, and the growth will develop anaerobically along the track of the needle.

LIBORIUS'S METHODS.

- (a) Fill a test-tube three-quarters full of gelatine or agar, sterilize, and place in a vessel of boiling water for ten minutes to expel all air from it. Cool the medium rapidly in ice-water; when between 30° and 40° C. and still fluid, inoculate, solidify rapidly, and seal up the tube in the flame. The anaerobic bacteria develop only in the lower layers of the medium.

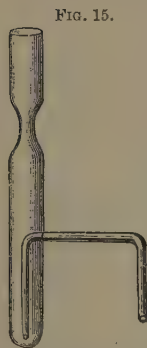


FIG. 15.

Liborius's Tube for
Anaerobic Cultures.

- (b) For this method a special tube is necessary (see Fig. 15). Hydrogen is passed through the side tube by means of the hydrogen apparatus (see Fig. 18), until all air is expelled. The contracted parts at the neck and the side tube are sealed in the flame. Owing to the small capacity of these tubes they give better results with fluid media. For the necessary precautions to be observed in using hydrogen see (p. 81).

FRAENKEL'S METHOD.

1. Prepare a tube in the same manner as for a plate or Esmarch roll-culture.

2. Replace the cotton by a sterile rubber stopper with two glass tubes passing through it, and plugged with cotton before sterilization. The tubes on the outside of the stopper are bent at right angles to the

long axis of the test-tube, and slightly drawn out in the flame. One of the tubes reaches within 0.5 cm. of the bottom of the tube, while the other is cut off level with the inside of the stopper. The hydrogen apparatus is attached to the end of the longest tube, and hydrogen is allowed to bubble through the tube until all air is expelled and its place taken by the hydrogen. The hydrogen must be passed through the gelatine at least ten to fifteen minutes, to ensure that all oxygen is expelled. The drawn out portions of the tubes are then sealed in the flame, and the protruding end of the rubber stopper painted with paraffin.

Before using the hydrogen apparatus test the hydrogen, and make sure it is free from oxygen, as follows: Fill an ordinary test-tube with water, close the mouth with the thumb, invert it, and place its mouth under water; remove the thumb, and the water will be kept in by atmospheric pressure. Conduct the hydrogen into the test-tube by



FIG. 16.—Kitasato's Flask for Anaerobic Cultures (Side and Front View).

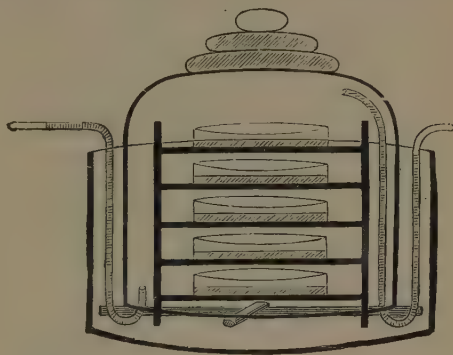


FIG. 17.—Botkin's Apparatus for Anaerobic Plate-Cultures.

means of a piece of rubber tubing, when the water in the tube will be replaced by gas. Hold a flame near the mouth of the test-tube, and if no explosion occurs the hydrogen is safe to use.

Kitasato devised a special flat flask for making anaerobic cultures in a hydrogen atmosphere (see Fig. 16). Kitasato and Weyl also suggest the addition of formic acid (0.3 to 0.5 per cent.), glucose (1.5 to 2 per cent.), or blue litmus-tincture (5 per cent. per volume), to the culture medium in addition to an atmosphere free from oxygen.

Botkin (1890) devised a simple apparatus for anaerobic plate-cultures (see Fig. 17). The plates or dishes are placed on a rack which stands in a dish containing liquid paraffin, this dish admitting a

weighted bell-jar. Leading in and out of the bell-jar are pieces of tubing, which serve to conduct the hydrogen or other gas used.

BUCHNER'S METHOD.

By this method the atmosphere is robbed of its oxygen by means of pyrogallic acid. Either oblique, roll, or stab-cultures are made in a test-tube which is placed inside a larger tube, having a brass wire, or better, sand, for the bottom of the smaller tube to rest in (see Fig. 19). One gramme of pyrogallic acid and 10 c.c. of $\frac{1}{10}$ th normal caustic potash solution* are put in the larger tube, which is quickly and tightly plugged with an india-rubber stopper. The oxygen is rapidly absorbed by the pyrogallic acid.

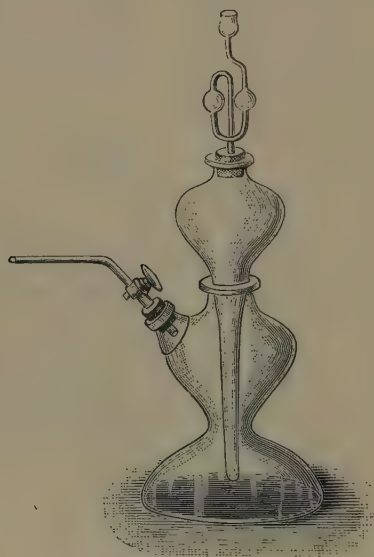


FIG. 18.—Kipp's Hydrogen Apparatus.



FIG. 19.—Buchner's Tube for Anaerobic Cultures.

ANAEROBIC CULTURES IN LIQUID MEDIA (KASPAREK'S METHOD).

A special flask is necessary for this method which is intended to be used when large quantities of bacterial products, etc., are required. The flask is provided with a small tube terminating in a bulb blown into its neck about $\frac{3}{8}$ of an inch above the top of the liquid medium. The flask is filled with bouillon almost to the neck, then 3 c.c. of liquid paraffin are added, and the whole is sterilized in the usual

* A normal solution of caustic potash contains as many grammes to the litre as the number of its molecular weight—56.1 grammes to the litre of water.

manner in the steam sterilizer. The heat expands the bouillon, and causes the paraffin to rise in the neck of the flask and overflow into the side tube and bulb. After sterilization is completed, a thin layer of the paraffin remains on the top of the bouillon, and prevents re-absorption of oxygen while the flask is cooling. Before inoculation it is necessary to pierce the thin paraffin film on the top of the bouillon. After inoculation heat the side bulb gently to melt the paraffin, and by slightly inclining the flask, allow the paraffin to flow out of the bulb into the neck of the flask above the bouillon. Upon hardening, the paraffin forms a stopper, and when the flask is placed in the incubator, the heat and gases generated in the culture cause this stopper to be pressed upwards into the constricted neck of the flask, thus entirely excluding the air.

CULTIVATION OF ANAEROBES ON SLANTED AGAR
(VOTTELER'S METHOD).

1. Take a wide tube containing slanted agar, and inoculate the surface after having poured off the water of condensation.
2. Place the tube upside down in a larger tube containing a solution of pyrogallic acid covered with liquid paraffin.
3. Pass hydrogen through the pyrogallic solution, seal with a mixture of paraffin wax and vaseline, and place in the incubator.

THE INCUBATOR.

Certain forms of bacteria develop at a higher temperature than others. Pathogenic or disease-producing organisms as a rule grow better at 37.5° C. than at lower temperatures, whereas the ordinary saprophytic forms develop almost at any temperature between 18° C. and 37.5° C. For the cultivation of pathogenic bacteria an apparatus known as a Thermostat or Incubator is used. The thermostat is provided with double walls, the space between being filled with water. It is provided with closely-fitting outer and inner doors, the inner being of glass, whereby the contents of the chamber may be inspected without actually opening it. The temperature within the incubator is kept constant by an automatic regulator, which prevents a fluctuation of more than 0.2° C. in the temperature of the air within the apparatus. A Koch's safety burner is generally used for heating, the safety attachment automatically turning off the gas supply, thus preventing

accidents should the flame be extinguished when no one is near (see Fig. 23).

Another form of incubator which is very generally used in England is Hearson's. A copy of this apparatus is sold in Germany under the name of the "Sartorius Thermostat." The original Hearson's (patent) apparatus is made for heating either with gas (Fig. 20) or a petroleum lamp (Fig. 22). The chamber of the incubator is surrounded by a double-walled copper receptacle which contains water, and is covered by a casing of pine to lessen loss of heat. The apparatus has an outer and inner door (Fig. 20) made of wood and glass respectively. At the

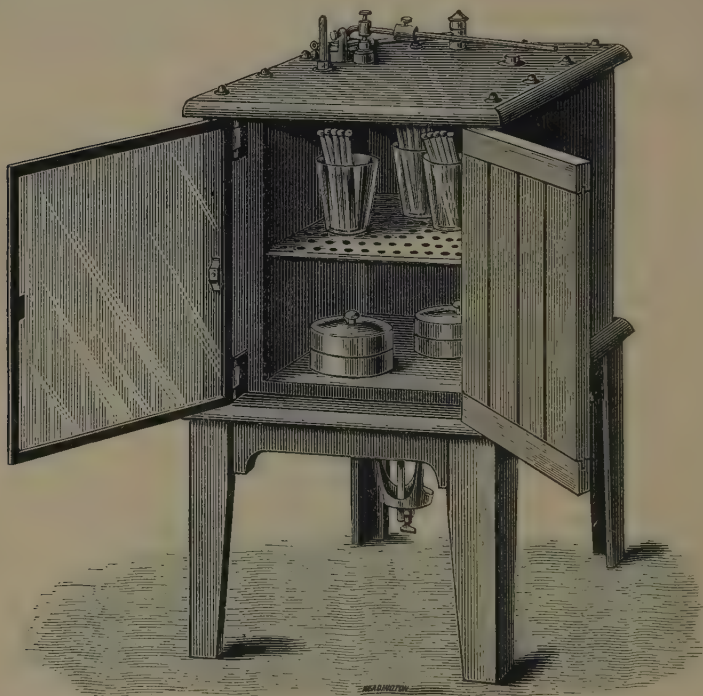


FIG. 20.—Hearson's Thermostat (for heating with gas).

top on the left and to the back of the apparatus (better seen in Fig. 22) is a small hermetically sealed metallic capsule (*S*) which contains a few drops of liquid having a boiling point at or near that at which it is desired to regulate the thermostat. A vertical needle rests upon the capsule, whilst above it enters into the socket end of the screw *P*. When used for gas the apparatus is supplied with the "Excelsior Gas

Valve," which is shown in Fig. 21. The gas flows into the valve at *A*, and out to the burner at *C*. At *B D* there is a lever pivoted to standards at *G*. The lever is acted upon by the capsule, through the vertical needle which enters the socket in screw *P*.

"The construction of the acting portion of this valve is such that, whenever the end *B* of the lever *B D* presses on the disc below the end *B*, the main supply of gas is entirely cut off. At such times, however, a very small portion of gas passes from *A* to *C*, through an aperture inside the valve, the size of which aperture can be adjusted by the screw-needle *S*, hence the gas-flame which burns in a little lantern below the incubator is never extinguished."

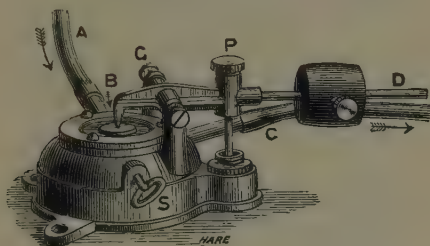


FIG. 21.—The Excelsior Gas Valve.

The lever *B D* is moved to and fro through the expansion and contraction of the capsule. A sliding weight runs on the lever-rod *D*, and serves for retarding the boiling-point of the fluid in the capsule, for by moving it along the lever away from *P* a greater pressure is exerted in the capsule. By the use of this weight a range of about 8° Fahr. is obtained with a single capsule. When set for a given temperature the apparatus does not vary usually more than half a degree, though the gas pressure and outside temperature may vary considerably.

The apparatus for heating with a lamp (Fig. 22) will be found useful for working in places where no gas can be obtained. In this apparatus the hot air and gases from the lamp *T* passes through the tube *L* which is immersed in the water receptacle. This heats the water. The cooled gases from the lamp pass out through a second chimney which discharges into the open air at a point behind, where the tube *L* enters the thermostat. The capsule *S* acts on the lever *D*, and this lowers or raises the damper *F*, which rests on the chimney *V*, when the apparatus gets too cold, thus permitting the heated gases to

pass through *L*. If the temperature of the chamber rises, the capsule expands, and causes the lever to raise the damper, whereby more or less hot air escapes through the top of the chimney.

Hearson and Co. also manufacture a thermostat in which a temperature below that of the surrounding air can be maintained. This apparatus is cooled by means of a small stream of cold water or by means of ice. This apparatus is constructed upon the same principles,

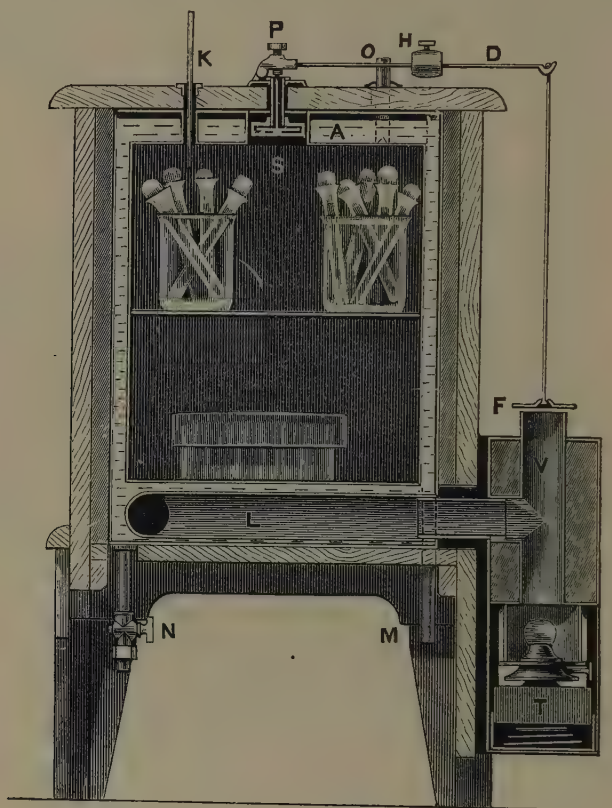


FIG. 22.—Hearson's Thermostat for heating with petroleum lamp (seen in section).

the movement of a capsule regulating the flow of the cold water through the apparatus. Such an apparatus is needed in hot weather or in the tropics, when lower temperatures are desired, more especially when gelatine is used for cultivation purposes. A detailed description of these thermostats accompanies each apparatus.

Fig. 23 represents a German thermostat, such as is found perhaps

most frequently in bacteriological laboratories. In a general way it is constructed upon the same plan as Fig. 20. The loss of heat is prevented by a layer of asbestos instead of wood, and it is provided with a

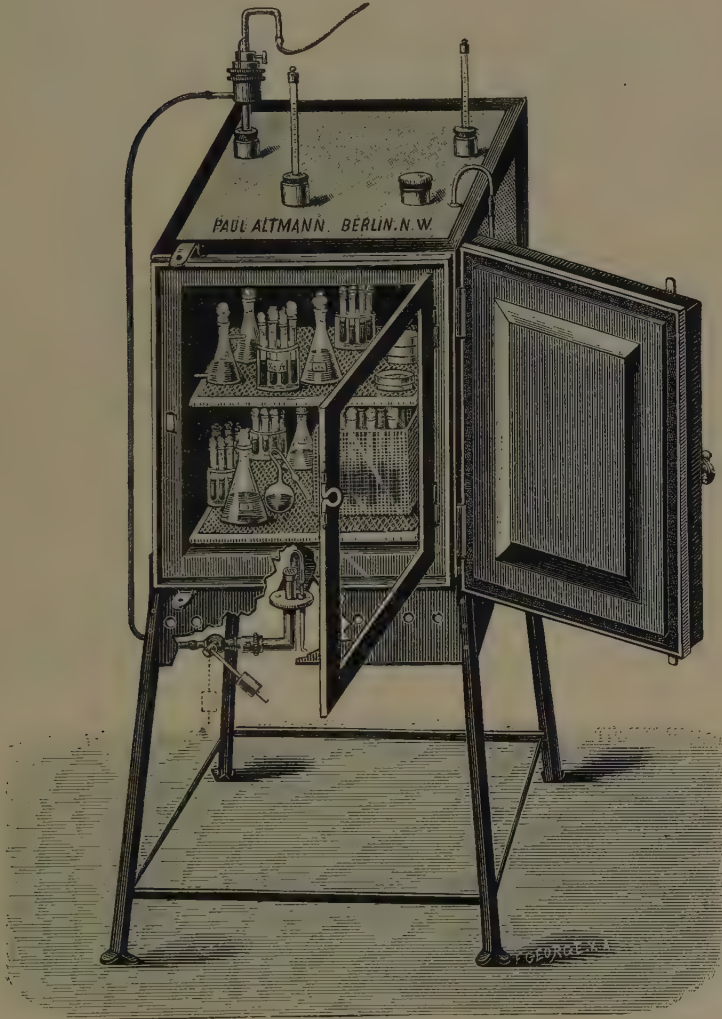


FIG. 23.—Incubator.

mercury thermo-regulator instead of with a capsule as in the Hearson model. The rise and fall of the column of mercury depending upon the fluctuations of temperature, regulates the flow of gas.

REACTIONS PRODUCED BY BACTERIA DURING THEIR GROWTH.

The reactions produced by many species of bacteria in the course of their development in culture-media may be of considerable specific value. These changes in some instances are so great that they can be detected by simple reagents, whilst in others they are so slight as to require the most delicate test for their demonstration. Some bacteria produce at one period of their life an alkaline and at another an acid reaction. This is seen in cultures of the diphtheria bacillus.

ACID AND ALKALI PRODUCTION.

Changes in reaction are best observed when a chemical substance, which does not interfere with the development of the organism, is added to the medium. In milk, to which litmus has been added (see p. 63), organisms producing alkali cause the blue colour to be intensified, whilst those producing acid change it to red. Others again bring about neither of these changes. Tincture of litmus can also be added to gelatine and agar-agar for the same purpose. Alcoholic solution of rosolic acid (see p. 62) is used with the same object as litmus.

In ordinary milk cultures, acids produced from milk-sugar by bacterial action upon the casein of the milk frequently bring about coagulation, but at times acids may be produced without resulting coagulation.

GAS PRODUCTION.

Gas production is associated with the growth of some bacteria, and is best seen in cultures grown in media containing 1 to 2 per cent. of grape-sugar. This gas production may be observed in various ways, the simplest being in so-called "shake-cultures" in agar or gelatine.

1. Liquefy the medium, and lower its temperature to a suitable degree for inoculation.
2. Inoculate the liquefied medium in the ordinary way with a small quantity of pure culture, and distribute the organisms equally.
3. Place the tube upright in ice-water and solidify the medium rapidly.
4. Place in the incubator.
5. In twenty-four to thirty-six hours, if the organism causes

fermentation of glucose, the medium will contain numerous small gas bubbles.

To determine the nature and amount of the gases formed, a fermentation tube or corresponding apparatus (Figs. 24 and 25) is necessary.

A fermentation tube in its simplest form is shown (Fig. 25), and consists of a tube bent at an acute angle, and closed at one end. A more useful form is that recommended by Smith (Fig. 24), which has

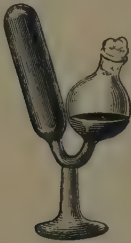


FIG. 24.—Smith's Fermentation Tube.



FIG. 25.—Dunbar's Fermentation Tube.

a bulb enlargement at one end, and is attached to a glass foot, so that it may stand upright. The tube is only used with fluid media. To use a Smith's fermentation tube proceed as follows :—

1. Pour fresh 2 per cent. grape-sugar bouillon into the bulb of the tube until it is half full.
2. Tilt the tube until the closed arm is nearly horizontal, so that the air in the arm may escape into the bulb and the fluid flow into the arm to take its place.
3. When the arm is completely filled, add enough of the liquid medium to cover the lowest expanded portion of the bulb, and close the opening of the bulb with a cotton plug.
4. Sterilize the tubes on three consecutive days by the usual method.

During the process of sterilization the tension of the vapour in the arm forces most of the fluid into the bulb. When the tube cools, the fluid returns again into the arm, except in a small space at the top, occupied by air originally dissolved in the liquid, and which was driven out by the heat. The air bubble must be tilted out after

each sterilization, and finally after the third sterilization the arm of the tube will be free from air.

After inoculation, the tubes are placed in the incubator, and the amount of gas collecting in the closed arm noted daily. The gas is usually found to consist of about one part by volume of carbonic acid and two parts by volume of an explosive gas, consisting largely of hydrogen. To determine the character of these gases Smith proceeds as follows :—

1. *CO₂ Production.*—The bulb is completely filled with a 2 per cent. solution of sodium hydroxide (NaOH), and closed tightly with the thumb. The fluid is thoroughly shaken with the gas, and allowed to flow to and fro from bulb to closed branch several times to insure intimate contact of the CO₂ with the alkali. Lastly, *before removing the thumb all the gas is allowed to collect in the closed branch*, so that none may escape when the thumb is removed. If CO₂ is present, a partial vacuum in the closed branch causes the fluid to rise suddenly when the thumb is removed. After allowing the layer of foam to subside somewhat, the space occupied by gas is again measured, and the difference between this amount and that measured before shaking with the sodium hydroxide solution gives the proportion of CO₂ absorbed.

2. *Other Gases.*—The explosive character of the residue is determined as follows : Replace the cotton plug, and allow the gas in the closed branch to flow into the bulb and mix with the air present there. The plug is removed, and a lighted match inserted into the mouth of the bulb. The intensity of the explosion varies with the amount of air present in the bulb.

METHOD OF DETECTING INDOL IN CULTURES OF BACTERIA.

1. Cultivate the organism for twenty-four to forty-eight hours at 37° C. in Dunham's peptone-solution (see p. 62), using four tubes kept under exactly the same conditions.

2. Apply the test as follows : Take two (control) tubes, each containing 7 c.c. of the peptone-solution, but *not inoculated*. To one add 10 drops of concentrated sulphuric acid, to the other 1 c.c. of 0.01 per cent. solution of sodium nitrite, and afterwards 10 drops of concentrated sulphuric acid. In five to ten minutes, if no *rose colour* appears, then *indol* is absent.

3. To two *inoculated* tubes add 10 drops of concentrated sulphuric

acid, and in five to ten minutes, if no rose colour appears, add 1 c.c. of the sodium nitrite solution; if no *rose colour* appears, then *indol* is absent.

When the *rose colour* appears with the addition of the concentrated sulphuric acid *alone*, then *indol* has been formed, and likewise a *reducing body*. When the *rose colour* appears only with the addition of *both* the concentrated sulphuric acid and the nitrite solution, then *indol* has been formed during the growth of the organism, but no *nitrites*.

BACTERIAL FILTERS.

A variety of filters have been used for bacteriological work, all of them being modifications or special adaptations of the well-known

FIG. 26.

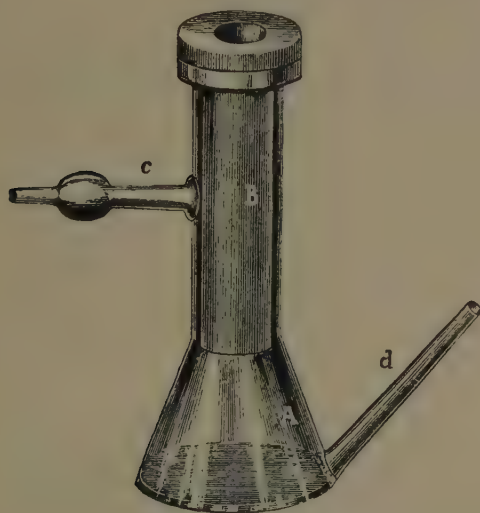


FIG. 27.



Reichel's Bacterial Filter.

Pasteur-Chamberland porcelain filter. An apparatus which is very generally used is that of Reichel. This consists of a glass flask (Fig. 26) and a porcelain filter (Fig. 27) which fits in the flask.

Method of using the Filter.—1. Place some cotton wadding in the tube at C, and also at D.

2. The filter, B, is sterilized for thirty minutes in the autoclave, and is then placed in the previously sterilized glass flask, or both are sterilized at once in the hot-air sterilizer, a circular piece of asbestos

being placed under the flange of the filter and the upper surface of the neck of the flask.

3. When the apparatus is removed from the sterilizer, a perforated rubber cap is placed over the top of the filter and flask, and the material to be filtered poured into the filter. A piece of rubber tubing with a pinch-cock is attached at *D*. The tube *C* communicates directly with the tube of a suction-pump. Novy* has devised a useful apparatus, by which both suction and pressure may be used in filtering. Bacterial filters are used for separating bacteria from fluid in which they may be suspended. This fluid may contain their soluble products. If proper precautions are taken the fluid which has been thus filtered remains sterile.

INOCULATION OF ANIMALS.

The animals usually employed in laboratories for inoculation purposes are white mice, house-mice, field-mice, rats, guinea-pigs, rabbits, and pigeons. Inoculations may be made subcutaneously, intravenously, into the great serous cavities, or into the anterior chamber of the eye, etc.

SUBCUTANEOUS INOCULATION.

1. Remove the hair or feathers, wash the skin with soap and water, and sterilize with corrosive sublimate solution 1 to 1000.

2. If a culture-fluid is to be inoculated this is injected by means of a sterilized hypodermic syringe.

3. If pieces of organs, or bacteria, earth, etc., are to be inoculated, a pocket is made in the skin. Take up the skin with the forceps, make a small incision with sterilized scissors, introduce a blunt pointed instrument and form a pocket under the skin. Hold the pocket open with sterilized forceps, introduce the material with the platinum loop into the pocket as far back as possible without touching the edges of the wound.

4. Draw the edges of the wound together, and, depending upon its character and size, allow it to remain, or stitch and cover it with a little iodoform collodion, or sear with a hot platinum needle. During the operation the animal must be held perfectly still. For the smaller animals many forms of holders are made, especially for mice, which are held in proper position for inoculation at the root of the tail. Guinea-

* *Centralbl. f. Bakteriöl.*, 1897, xxii., p. 337.

pigs, rabbits, and pigeons are best held by an assistant. Pigeons are generally inoculated in the pectoral muscles, mice at the root of the tail, while the other animals are generally inoculated in the abdominal wall, either to the right or left of the median line.

INTRAVENOUS INOCULATION.

In the rabbit this operation is generally performed into one of the veins of the ear, the most suitable vessel being the ramus lateralis posterior of the vena auricularis posterior, a very fine, delicate vessel running along the posterior margin of the ear; and being firmly fixed in the dense surrounding tissue, it does not roll about when the needle is being inserted. The largest branch of the vena auricularis posterior is the central branch, or ramus anterior, but the insertion of a needle into this vessel is less readily accomplished.

If there is only a little blood in the ear, pressure at the base of the ear, combined with gentle friction of the part, will cause stasis of blood and distension of the vessels, rendering them more visible. The injection is always made from the dorsal surface of the ear. Needles employed for intravenous injection must have a perfectly flat slanting surface free from curvature. Care must be taken that no air is injected. The syringe and needles are sterilized before use in the steam sterilizer or in boiling water. There are a variety of patterns of syringes in use none of them being entirely satisfactory. The most practical ones are provided with asbestos or polished metal pistons which withstand sterilization.

INOCULATION INTO THE LYMPHATIC CIRCULATION.

Bacteria may be made to find their way into the lymphatics by way of the testicles, the hypodermic needle being made to penetrate into the substance of the testicle.

INTRAPLEURAL AND INTRAPERITONEAL INOCULATIONS.

To inject fluid into the peritoneum, sterilize the skin, and plunge the needle direct into the peritoneal cavity. There is not much danger of wounding the intestines and other viscera when the inoculation is made close to the median line, half-way between the sternum and the symphysis pubis. A curved needle may be used, with an opening on the convex side some distance from the point; but a good operator can use a straight needle.

When solid substances, bits of tissue, etc., are to be introduced, the operation must be conducted on the lines of a laparotomy, as follows :—

1. Shave the hair from a small area over the median line, wash the skin with soap and water, rinse with water, rub with alcohol, and finally sterilize with corrosive sublimate solution 1 to 1000.

2. Make a longitudinal incision about 2 cm. long, close to the median line, through the skin and down to the fascia.

3. Two subcutaneous sutures, as employed by Halsted, are introduced transversely to the line of incision about 1 cm. apart, and their ends left loose. These sutures do not pass through the skin proper, but are introduced into the subcutaneous tissues, passing into the abdominal cavity and out again, entering at one side of the line of incision and leaving at the other.

4. The remaining tissues are now cut through, and the bit of tissue deposited in the peritoneal cavity (under aseptic precautions), the edges of the incision are closed tightly and evenly by drawing and tying the sutures. The line of incision is dressed with iodoform collodion.

All instruments, sutures, ligatures, etc., to be used in the operation are sterilized, either in the steam sterilizer, or boiled in a 2 per cent. solution of sodium carbonate for ten minutes, and the operator's hands cleansed with disinfecting solution. The material placed in the abdominal cavity must also be handled with sterilized instruments.

Inoculation into the pleural cavity is seldom practised, as it is very difficult to enter the pleural cavity without injuring the lung.

INOCULATION INTO THE ANTERIOR CHAMBER OF THE EYE.

This operation is usually performed on rabbits. The animal should be held firmly in position by means of a holder. Before proceeding to operate, a few drops of 2 per cent. cocaine solution are dropped into the eye.

A puncture is made through the cornea just in front of its junction with the sclerotic, the knife passing into the anterior chamber in a plane parallel to that of the iris, when the aqueous humour flows out. Introduce the bit of tissue with fine sterilized forceps or a platinum loop through the opening upon the iris, where it remains. Cohnheim employed this method in demonstrating the infectious nature of tuberculous tissues, tubercular iritis being a constant

result when tubercular matter was introduced into the anterior chamber of the rabbit's eye.

SUBDURAL AND INTRACEREBRAL INOCULATION.

These methods are most frequently used in work on rabies.

1. Remove the brain of the suspected animal under aseptic precautions, as soon as possible after death.

2. Place a small piece of the brain or spinal cord in a sterile mortar, and grind it up thoroughly with a few c.c. of sterile water or bouillon.

3. Etherize a rabbit, clip the hair from the head between the eye and ear, wash the skin, and disinfect with 1 to 1000 sublimate solution.

4. A longitudinal incision is made through the skin and subcutaneous tissue in the median line, while a crucial incision is made through the periosteum on one side of the median line, thus avoiding hæmorrhage from the longitudinal sinus. The periosteum is then reflected or pushed back. Cut out a piece of bone about $\frac{1}{3}$ of an inch in diameter with a trephine, exposing the dura mater. The opening should be made at a point along the line joining the posterior margins of the two orbits.

5. Inject a drop or more of infectious material beneath the dura mater with a hypodermic syringe. Replace the periosteum, suture the skin, disinfect, dry, and apply some iodoform collodion. A piece of the suspected tissue may also be introduced directly under the dura mater. The inoculation-wound heals rapidly.

Symptoms of rabies may appear in fifteen to thirty days, sometimes they occur before fifteen days, at other times later, *i.e.*, after one to three months.

Leclainche and Morel* have found intracerebral inoculation simpler, quicker, and quite as effective in working with rabies. Instead of using a trephine, a drill 2 mm. in diameter is used for cutting through the bone. The hypodermic needle is then pushed outwards and forwards into the brain to the depth of 1 cm. and about 0.1 c.c. of the emulsion injected.

OBSERVATION OF ANIMALS AFTER INOCULATION.

Animals which serve for experiments, especially with pathogenic bacteria, require to be kept in isolated cages. Whereas mice can be kept in tall wide-mouthed bottles covered with weighted wire-gauze

* *Ann. de l'Inst. Pasteur*, 1899, xiii., p. 513.

tops, larger animals should be kept in cages which are entirely made of metal, so that they can be readily cleaned and disinfected. Such

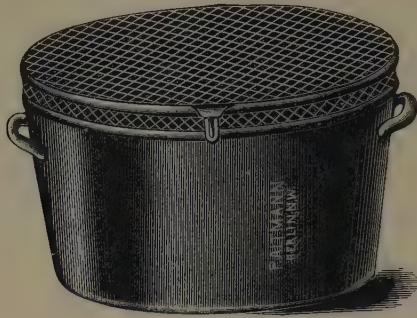


FIG. 28.

cages are made of various sizes, according to the size of the animals they are intended to contain. Figs. 28 and 29 show cages suitable for guinea-pigs and rabbits.

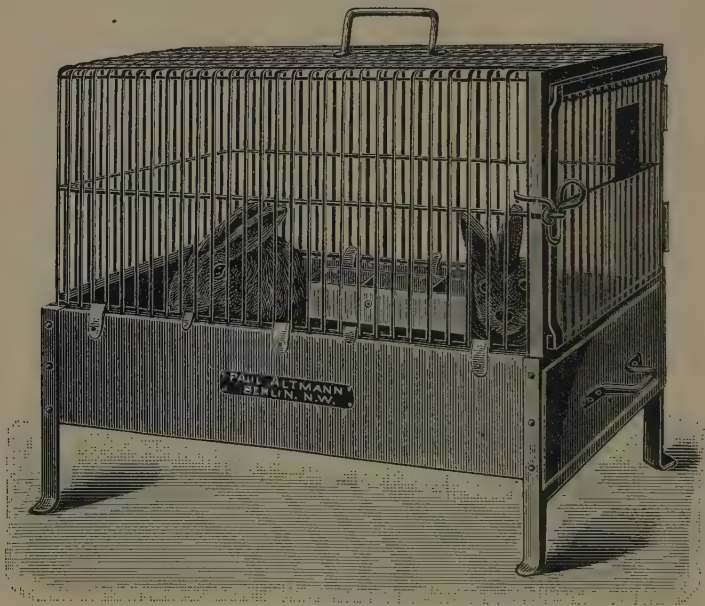


FIG. 29.

Inoculated animals should be kept under constant observation, and the following conditions noted :--

1. Temperature.

2. ~~Loss of weight.~~
3. Peculiarities of attitude.
4. Loss of appetite.
5. Condition of the coat or hair.
6. The condition of the secretions from the air passages, conjunctiva, kidneys, the occurrence of diarrhœa or hæmorrhage from the bowels.
7. The condition at the seat of inoculation.

When an animal dies in two to four days after inoculation, evidence of either acute or toxic processes will be found. When the inoculation produces chronic disease the animal may be under supervision for weeks. It should be weighed daily at the same hour; the temperature should be taken at the rectum (the thermometer being pushed past the sphincter). Too much stress need not be laid upon moderate and sudden daily fluctuations in either temperature or weight, as normal animals when confined in cages and fed regularly present striking temporary gains and losses in weight, and inexplicable rises and falls of temperature, often of as much as a degree from one day to another.

AUTOPSIES ON ANIMALS.

Perform the autopsy as soon as possible after death. When delay cannot be avoided, place the animal in the ice-chest until such time as is convenient.

1. Place the animal belly upwards upon a board of suitable size, and tack the four legs fast to the board.

2. Wash the surface of the thorax and abdomen with corrosive sublimate solution; make an incision through the skin at the pubis; introducing one blade of the scissors, and extend the incision as far as the chin.

3. Dissect the skin away from the abdomen, thorax, axillary, inguinal, and cervical regions, and fore and hind legs, and pin it to the board as far as possible from the thorax and abdomen. It is from the skin that the chances of contamination are greatest.

All incisions from now on are made with sterilized instruments.

4. Take an ordinary potato-knife, heat it, and sear the tissues along and across the linea alba, making two sterilized tracks through which the abdomen may be opened by crucial incisions; two lines may also be seared along the sides of the thorax.

5. Make a longitudinal incision from the sternum to the symphysis pubis with heated scissors, the abdominal wall being held up with sterilized forceps, or a hook, to prevent the internal viscera being injured. Transverse incisions are made in a similar manner.

6. Cut through the ribs with strong sterilized scissors along the sterilized tracks on the sides of the thorax, when the whole anterior wall of the thorax is easily lifted and entirely removed by severing the connections with the diaphragm, etc.

7. When the thoracic and abdominal cavities are fully exposed a careful examination of the organs and surroundings is made without disturbing them.

8. Plate, Petri-dish, or roll-cultures, are prepared from the blood, liver, spleen, kidneys, etc., and any exudates present. To obtain cultures from organs proceed as follows:—

(a) Heat a scalpel and sear a small part of the surface of the organ from which the cultures are to be made.

(b) Heat the scalpel again and penetrate the capsule of the organ with the point, and through the opening insert a stout sterilized platinum loop, push it into the tissues, twist it around, and obtain enough material from the deeper parts of the organ to make the culture.

In making cultures from resisting tissues Nuttall's platinum spear can be used to advantage. It is a piece of heavy platinum with a spear-head at one end perforated with a small hole, the other end being attached to a metal holder. When heated it can be readily thrust into the densest of the soft tissues, and when withdrawn after twisting it about, sufficient material will be found in the eye of the spear-head for purposes of direct examination or for the preparation of cultures.

Cultures from blood are usually made from one of the heart cavities, the surface being seared with a hot knife before opening. After culture material is obtained, cover-glass specimens may be prepared from each organ, exudates, etc.

Small pieces of each organ may be also preserved for microscopic examination (see p. 37).

When the autopsy is finished the cadaver of the animal should be burned and the instruments used sterilized (see p. 11). If a large post-mortem board has been used disinfect it with sublimate solution

1 to 1000. Small boards can be exposed to the flame of a Bunsen burner. Used cover-glasses and other infectious material should also be sterilized if of no further use.

METHODS OF EXAMINING AIR.

ORDINARY METHOD.

1. Liquefy 10 c.c. of nutrient gelatine or agar, pour it upon a sterile plate or into a Petri-dish, and allow it to cool.
2. Remove the cover of the dish or plate, leaving the gelatine exposed for one hour.
3. Replace the cover, set aside, and examine the colonies which develop upon the medium.

This method yields only qualitative results.

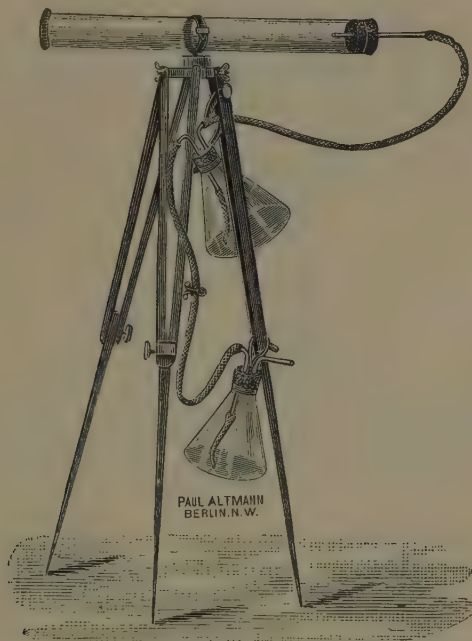


FIG. 30.—Hesse's Apparatus for Examining Air.

HESSE'S METHOD (FIG. 30).

1. About 50 c.c. of sterile liquefied gelatine are introduced into a sterilized glass tube 70 cm. long and 3 to 5 cm. in diameter. The tube is then rotated as in the roll-culture process (see p. 74), the

gelatine being allowed to harden upon the inner surface of the tube. One end of the cylinder is closed by two elastic caps, the inner one having a central orifice; the other end is closed with a rubber stopper, through which a glass tube (plugged with cotton wool) passes.

2. The tube is placed on a tripod. The small tube is connected with an aspirator capable of drawing air through the cylinder at a velocity of about half a litre per minute.

3. Before the aspirator begins to act, the outer rubber cap at the opposite end of the large tube is removed. Any germs passing into the cylinder adhere to the gelatine upon which they subsequently form colonies.

PETRI'S METHOD.

By this method a greater quantity of air can be tested than by Hesse's method.

1. Prepare a glass tube 9 cm. long and 1.5 cm. in diameter, containing two filters of fine sand separated from each other by wire-gauze netting, the ends of the tube being plugged with cotton.

2. After the tube is sterilized in the hot-air sterilizer, it is placed vertically in a holder, and an end of the tube is attached to an air-pump (Fig. 31) capable of aspirating 5 or more litres of air per minute. The plug at the distal end of the tube is removed, and the pump set in motion.

3. The air-pump is worked until the required amount of air has passed through the filter.

4. The filter at the distal end of the tube is removed by shaking the sand out and mixing it with 10 c.c. of sterilized liquefied gelatine, which is poured into a sterile Petri-dish. The sand-filter at the proximal end of the tube is used as a control, and should yield a negative result when plated.

Powdered glass can be substituted for sand, and is more satisfactory, as any developing colonies can be more readily observed.

SEDGEWICK-TUCKER METHOD.

In this method a soluble filter is used. The air to be examined is aspirated through a glass vessel termed an "aëroscope," a cylindrical vessel (15 cm. long by 4.5 cm. in diameter) ending at one extremity in a bottle-like neck (2.5 cm. long by 2.5 cm. wide), and at the other in a glass tube (15 cm. long, with an internal diameter of 0.5 cm.).

Wire gauze is pushed into the narrow tube and serves to support granulated sugar which serves as a filter. The apparatus is plugged with cotton at both apertures and sterilized in the hot air chamber prior to the introduction of the sugar. After it has cooled the apparatus is held narrow tube downwards and the sugar shaken into the tube until it forms a column 10 cm. high above the wire gauze. The apparatus is now sterilized again for some hours at 120° C. When about to be used the tube is placed in an upright position, narrow tube downwards, the lower end being connected with an aspirator—a bottle

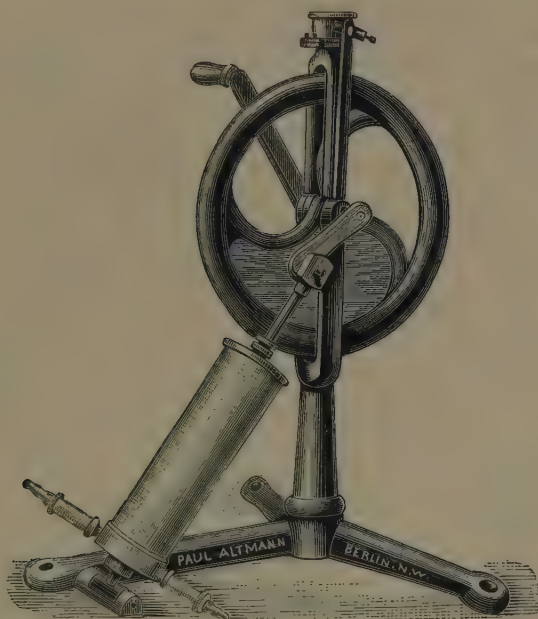


FIG. 81.—Air-pump for use in Petri's Method.

of known capacity filled with water and emptying itself can be used, or a pump with interposed gasometer. The cotton plug at the upper end is removed and the air filtered through the sugar. When sufficient air has been filtered, the cotton plug is replaced and the sugar shaken into the enlarged portion of the *aëroscope*, after which about 20 c.c. of sterile melted nutrient gelatine is poured into the cylinder (which is held horizontally) by means of a funnel provided with a bent neck. The sugar is dissolved by the gelatine, which is rolled upon the inside of the cylinder after the manner of a roll-culture.

A useful modification of this method, which requires no special apparatus, consists in placing the granulated sugar in glass tubes about 0.5 cm. in diameter, which are drawn out to a point and sealed at one end, being closed by cotton at the other extremity. The filled tubes are sterilized as in the preceding case. When about to be used the tube is mounted point up in a holder, the cotton-plugged end is connected with an aspirator, and the sealed end is broken off after being sterilized. After the air has passed through the filter the sugar is shaken into melted gelatine and plated in the usual way.

METHODS OF EXAMINING WATER.

When water is collected for bacteriological examination, every care should be taken to exclude contamination from other sources. Samples taken from running waters, or the water of a lake, pond, or well, should be collected at a depth usually of a foot or more beneath the surface. Water taken from a tap should be collected after it has been allowed to run for about a quarter of an hour. The vessels in which the sample is collected for analysis should be thoroughly cleaned and sterilized by dry heat. Water may be collected in various ways depending upon the source. Samples may be taken by means of sterilized Erlenmeyer flasks, by means of sterilized bulbs provided with a tube which is sealed in the flame after the air has been expelled, the sealed end being broken off beneath the surface of the water, etc.

For obtaining samples at considerable or specified depths a Miquel's or similar apparatus can be used. It consists of a glass vessel of about 50 c.c. capacity with a neck drawn out to a fine point and bent, and so arranged that when it is lowered to the required depth the neck of the flask can be broken off by pulling a copper wire previously attached to the bent neck of the flask. When the neck is broken the water rushes into the sterilized and vacuous vessel. The apparatus is then drawn up; the neck of the flask is sealed in the flame, if the examination is not made immediately.

The accompanying figure (Fig. 32) shows the Sclavo-Czaplewski apparatus ready to be lowered into the water from which a sample is to be taken for analysis. The apparatus is lowered by means of a graduated string wound about the spool (*a*). The glass receptacle is mounted in a brass holder (*c*), with its sealed capillary tube resting upon the metal plate in the centre of which the string terminates. The

weight (*a*) causes the apparatus to sink vertically into the water. When it has reached the desired depth, the weight (*b*) is released from the hand, and, sliding downward along the string, breaks off the sealed end of the tube against the metal holder on which it rests. The water then rushes into the vacuous tube, which rapidly fills itself. The apparatus is now drawn up to the surface, and the capillary tube is sealed in the flame.

The samples should be examined if possible very soon after they are taken, at latest within two hours. When examination is delayed, place the sample in the ice-box. Before testing a sample, shake the flask, as an equal distribution of the germs is necessary for an average result.

Water analysis should always be made on the spot, when possible, as during transportation, unless packed in ice, a multiplication of the organisms in the sample takes place. The following are the necessary articles for a *Transportation Case for Analysis on the Spot*:—

- 4 sterilized Erlenmeyer flasks to obtain the samples; 1 thermometer; 1 spirit lamp; 12 sterilized Petri-dishes in special box (see p. 8); 12 tubes of sterile nutrient gelatine; 15 sterilized water pipettes in three cases; 1 folding tripod; 1 notebook; 1 pencil for writing on glass, etc.; and 1 towel.

The melted gelatine is inoculated and poured into the Petri-dishes, and after it has been allowed to solidify, the cultures are transported to the laboratory.

QUANTITATIVE METHOD.

Transfer with a sterilized capillary pipette 1 c.c., $\frac{1}{2}$ c.c., or $\frac{1}{4}$ c.c. of the water to be examined into a tube containing 10 c.c. of liquefied sterilized nutrient gelatine. Mix, and pour the contents of the tube on a sterile glass plate, and proceed as described on p. 77.

KOCH'S METHOD FOR DEMONSTRATING THE VIBRIO CHOLERÆ ASIATICÆ IN WATER.

1. Take 100 c.c. of the suspected water, and mix it with 5 c.c. of

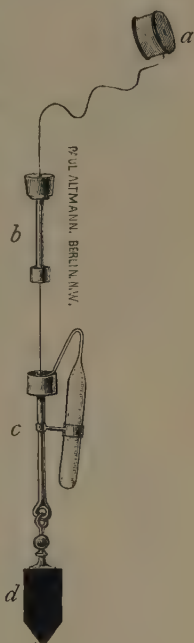


FIG. 32.

sterile 20 per cent. peptone and chloride of sodium solution (p. 62), make alkaline, and place in the incubator at 37° C.

2. When cholera spirilla are present they develop, and are found in ten to twelve hours on the surface of the fluid, and can be further investigated and identified.

ELSNER'S METHOD FOR BACILLUS TYPHI ABDOMINALIS.

Take some potato gelatine (see p. 61), and shortly before use add 1 per cent. of iodide of potash; after adding the suspected water, prepare plate-cultures according to the ordinary method. This method is used for the *B. typhi abdominalis* and the *B. coli communis*. Of the two the latter grows more vigorously in this medium, and in forty-eight hours produces dull brown colonies, while the typhoid bacillus produces bright watery drop-like colonies. The method, however, is not absolutely reliable.

METHOD OF EXAMINING WATER FOR THE BACILLUS TYPHI ABDOMINALIS AND BACILLUS COLI COMMUNIS.

1. Take 500 to 1000 c.c. of the suspected water and filter through a sterile Reichel filter (see p. 91).

2. Pour 10 c.c. of the sterile filtered water into a sterile cotton-plugged flask.

3. Remove the filter and scrub its surface with a sterile tooth-brush, by this means removing the micro-organisms deposited upon it. The sterile water in the flask (2) is inoculated with the material from the brush and the cotton plug replaced.

4. Melt several tubes of carbolic acid gelatine in the water-bath, and with a sterilized pipette add to each tube .005 to 0.5 c.c. of the bacterial mixture from the flask, and prepare a series of plate-cultures in the ordinary manner. Place the cultures in the cool incubator, and prepare sub-cultures from any colonies developing on the plate. The growth of large numbers of liquefying organisms is prevented by the carbolic acid in the gelatine.

QUICK METHOD OF DEMONSTRATING THE PRESENCE OF PATHOGENIC GERMS IN WATER.

1. Mix 100 c.c. of the suspected water with 5 c.c. of 20 per cent. peptone chloride of sodium solution (see p. 62), and place for twenty-four hours at 37° C. in the incubator.

2. Inoculate a guinea-pig with 1 c.c. of the mixture intraperitoneally, and if pathogenic organisms are present, the animal dies, and the organs, exudates, blood, etc., can be further examined.
3. If the water is pure, the guinea-pig remains alive.

SMITH'S METHOD OF ISOLATING INTESTINAL BACTERIA FROM A WATER SUPPLY.

1. Add one, two, or three drops of the suspected water to fermentation-tubes containing two per cent. grape-sugar bouillon.
2. Place at 37° C., and if at the end of thirty-six to forty-eight hours gas accumulates in the tube, then intestinal bacteria are present, as ordinary water bacteria do not flourish in this medium.
3. For further identification isolate the gas-forming organisms by the ordinary plate-culture method.

When the colonies which have developed on a plate are too numerous to be counted with Wolffhügel's apparatus (p. 77), the plate is examined with a low power and Ehrlich's eye-piece diaphragm.

METHODS OF EXAMINING SOIL.

Samples of soil gathered from the surface may be broken up with sterile implements and added directly to nutrient media, or

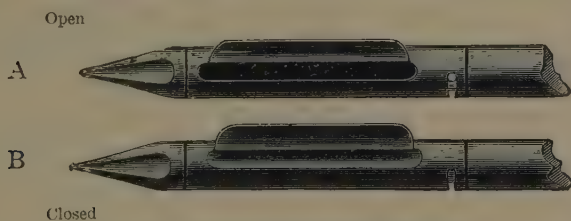


FIG. 33.—Fränkel's Earth-Borer.

better still, they are shaken up with water, and the water containing the germs is used for inoculating the culture media. In examining soil, different methods must be used according to the nature of the organisms which are to be isolated, some of these organisms being anaërobic, others aërobic, whilst nitrifying bacteria, etc., require specially constituted media (p. 71). When it is desired to examine the deeper layers of the soil a borer must be used. Fränkel's borer (Fig. 33) is usually employed for this purpose, being previously sterilized by heat. As long as the borer is turned to the right, the flange which

projects to one side keeps the collecting chamber closed, but when the borer is turned to the left, the flange remains stationary, whilst the borer turns, thus opening the chamber, within which the earth collects. This reversing in the rotation of the borer can be done at any desired depth. When the sample has been collected, the borer is drawn out, and a quantity of soil measured out by means of a sterilized platinum spoon into the media, or into the water used for inoculating the media. It is best to use plates or Petri-dishes for cultures from earth, roll-cultures being unsuitable on account of the number of liquifying organisms frequently present in earth.

The organisms most commonly encountered in earth which are of interest to medical men, are *B. tetani*, *B. œdematis maligni*, *B. coli*, *B. enteritidis sporogenes* (Klein). Nitrifying bacteria are found in the more superficial layers of the soil.

METHOD OF EXAMINING UNSOUND MEAT.

1. Feed mice or rats with some of the meat and watch the effect. If they die, prepare plate-cultures from the organs and heart's blood.

2. Crush some of the meat in sterile bouillon or normal salt solution and inoculate the extract into mice, rabbits, guinea-pigs, or other animals. Some should be inoculated subcutaneously, others intra-peritoneally. If the animals die, prepare cultures (as stated under 1).

3. Prepare plate-cultures from the meat extract, and cultivate aërobically and anaërobically.

4. Isolate any developing organisms, and test their pathogenic action on animals by means of feeding and inoculation experiments.

METHOD OF EXAMINING ICE CREAM.

1. Melt 100 c.c. of the ice cream in a sterile beaker at 38° C. and add 500 c.c. of sterilized water.

2. Prepare plate-cultures with ordinary gelatine, and also with carbolic acid gelatine, inoculating the tubes with quantities varying from .005 to .05 c.c.

3. Place the plates at room temperature, and count the developing colonies by the usual methods. For demonstrating the presence of *B. typhi* and *B. coli*, use carbolic acid gelatine media.

METHOD OF EXAMINING A DIPHTHERITIC MEMBRANE.

1. Wash the membrane in sterile saline solution. Cut off a small piece and break it up in the salt solution with a sterile glass rod.
2. Dip a platinum loop previously sterilized in the flame in the suspension, and inoculate three tubes containing Löffler's blood-serum media, as follows: Make three parallel streaks on the surface of the medium in the first tube. Without recharging the loop make three similar streaks on the surface of the medium in the second and third tubes.
3. Place the inoculated tubes in the incubator at 35° to 37° C.
4. Examine next day by the ordinary cover-glass methods (see p. 17), and stain with the special stains for the diphtheria-bacillus (see p. 23).

POINTS TO BE OBSERVED IN DESCRIBING AN ORGANISM—(ABBOTT).

1. Its source: air, water, or soil. If found in the animal body, is it normally present, or only under pathological conditions?
2. Its form, size, mode of development, occurrence of involution forms, or other variations in morphology. Grouping: in pairs, chains, clumps, zooglææ; presence of capsules; development and germination of spores; arrangement of flagella.
3. Staining peculiarities—especially its reactions with Gram's stain, and peculiar or irregular modes of staining.
4. Motility—to be determined on fresh cultures and on cultures in different media.
5. Its relation to oxygen—Is it aërobic, anaërobic, or facultative? In the presence of what gases does it develop: carbonic acid, hydrogen, etc.
6. Both the macroscopic and microscopic appearance of its colonies on nutrient gelatine and on nutrient agar.
7. The appearance of its growth in stab and slant cultures, on gelatine, agar, blood-serum, and on potato.
8. The character of its growth in fluid media: bouillon, milk, litmus-milk, rosolic acid peptone solution, glucose bouillon.
9. Does it grow best in acid, alkaline, or neutral media?
10. Is the normal reaction of the medium altered by its growth? Is its growth accompanied by the production of indol? Is the indol associated with the coincident production of nitrites?

11. Is its growth accompanied by the production of gas, as evidenced by the appearance of gas bubbles in the media, both in media containing fermentable sugars and those from which these bodies are absent? When cultivated in sugar-bouillon in the fermentation-tube, what gases are evolved under known conditions? How much of this gas is carbonic acid and how much is explosive?

12. At what temperature does it thrive best, and what is the lowest and highest temperature at which it will develop? What is its thermal death-point, both by steam and dry-air methods of determining this point?

13. What is its behaviour when exposed to chemical disinfectants and antiseptics? Does it withstand drying and other injurious influences, both in the vegetative and spore stages? The germicidal value of the blood-serum of different animals may also be tried upon it.

14. Its pathogenic powers—modes of inoculation by which these are demonstrated; quantity of material used in inoculation; duration of the disease and its symptoms; lesions produced, and distribution of the bacteria in the inoculated animal; which animals are susceptible and which immune, and the character of its pathogenic activities? Variations in virulence, and the probable cause to which they are due. Can they be produced artificially and at will?

15. The detection of specific, toxic, and immunizing products of its growth.

16. Its behaviour when exposed to the influence of blood-serum of animals immunized against it; also its behaviour when mixed with serum from an animal in the height of infection by it. Are the relations between the organism and the serum constant and specific?

PART III.

Special Bacteriology.

BACTERIA FOUND IN INFLAMMATION AND SUPPURATION.

THE bacteria most commonly associated with ordinary inflammatory and suppurative processes are :—

1. The pyogenic cocci, staphylococci, streptococci, pneumococci, etc.
2. The *Bacillus coli communis* and allied bacilli.
3. Friedländer's pneumobacillus is rarely present.
4. *Bacillus pyocyaneus*.

STAPHYLOCOCCUS PYOGENES AUREUS.

Microscopical Appearances.—Micrococci from 0.7 to 1.2 μ in diameter, usually arranged together like bunches of grapes. (See Fig. 34.)

Motility.—Non-motile.

Staining Reactions.—They are easily stained with all the basic anilin dyes, and by the Gram and Claudius methods.

Biological Characters.—Facultative anaërobe, producing yellow pigment only in the presence of oxygen. The minimum temperature for growth is 6° C., maximum 44° C., optimum 34° to 38° C.

On Gelatine Plates.—Examined under a low power, staphylococci at first form coarsely granular greyish-white colonies, with sharply defined borders; later the colonies assume a yellow colour and quickly liquefy the gelatine.

In Gelatine Stab-Cultures.—The development takes place along the whole length of the puncture, with accompanying liquefaction of the medium, the growth resembling a stocking.

On Agar Stroke-Cultures.—It forms a moist, shiny, gold-coloured elevated growth, a similar growth occurring on potatoes.

Bouillon becomes densely clouded, and a yellow sediment forms.

Milk is coagulated.

It is chiefly lactic acid which is formed in milk and bouillon.

Vitality.—Cultures remain alive for one year. They are killed in a short time in the steam sterilizer. On silk threads saturated with pus containing staphylococci, and dried, the germs are killed by 2 to 3 per cent. carbolic acid in five minutes.

Pathogenesis.—Cutaneous inoculation is ineffective, but sub-cutaneous injection produces local abscess in mice, guinea-pigs, and rabbits; and intravenous injection in rabbits may cause pyæmia.

Other Staphylococci are :—

STAPHYLOCOCCUS PYOGENES ALBUS.

This coccus is identical with the aureus, excepting that it forms no golden pigment.

STAPHYLOCOCCUS PYOGENES CITREUS.

This coccus produces a citron-yellow pigment, but in other respects resembles the aureus.

STAPHYLOCOCCUS CEREUS ALBUS AND CEREUS FLAVUS

Are seldom found, but are characterised by not liquefying gelatine media; the one exhibits a waxy white pigment, the other a waxy yellow pigment.

DIFFERENTIAL TABLE.

<i>Gelatine is liquefied.</i>	<i>Gelatine not liquefied.</i>
1. Staphyl. pyog. aureus.	1. Staphyl. cereus flavus.
2. " citreus.	2. " griseus.
3. " rosaceus.	3. " albus.
4. " albus.	

The staphylococcus pyogenes aureus is found in furunculosis, carbuncles, acute abscesses, circumscribed phlegmons of the skin, impetigo, sycosis, blepharo-adenitis, conjunctivitis phlyctenulosa, acute infectious osteomyelitis, suppuration of lymph-glands, empyema, articular and bursal suppuration, tonsillar abscesses, mammary abscesses, suppuration of the parotid, idiopathic cerebro-spinal

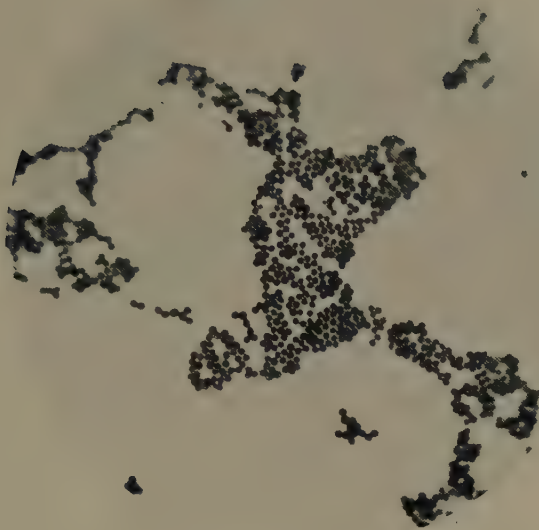


FIG. 34.—*Staphylococcus pyogenes aureus*. Agar culture. Claudius stain. $\times 1000$.



FIG. 35.—*Streptococcus erysipielatos*. Cover-glass specimen from bouillon-culture. Claudius stain. $\times 1000$.

meningitis, strumitis, and suppurative peripleuritis. The *Staphylococcus albus* is also often present in these infections—mixed infection being frequent.

STREPTOCOCCUS PYOGENES.

Microscopical Appearances.—Micrococci 0.3 to 1 μ in diameter, arranged in more or less long chains.

Motility.—Non-motile.

Staining Reactions.—Easily stained with all the basic anilin dyes, and by the Gram and Claudius methods.

Biological Characters.—Facultative anaërobe, the optimum temperature for its growth being 30° to 37° C. ; it also grows at room temperature.

On Gelatine Plates it develops in the form of small white granular colonies which do not liquefy the gelatine. Under a high power chains can be observed projecting from the sides of the colonies.

In Gelatine Stab-Cultures the growth is not confluent, but isolated colonies occur along the track of the needle. A similar growth occurs in agar stab-cultures.

Bouillon, which is an excellent medium for streptococci, is not clouded throughout, but a delicate flaky deposit is formed on the bottom and sides of the tube.

On Potatoes the growth is extremely scanty.

Milk is coagulated.

Vitality.—In cultures the streptococcus dies much sooner than the staphylococcus, only living about four months. In order to keep streptococcus cultures virulent, Petruschky uses gelatine, transplants every five days, and keeps the cultures in the ice-chest.

Pathogenesis.—Material containing streptococci, when rubbed on an abraded surface on a rabbit's ear, causes an erysipelatous inflammation. When inoculated into mice or rabbits septicæmia results, with or without local abscess. Intravenous injection causes septicæmia. Many suppurative processes spread by means of the lymphatics leading to lymphangitis and lymphadenitis. Rosenbach first obtained pure cultures of the *Streptococcus pyogenes* from such cases. It is also found in pyæmia in severe forms of arthritis, acute endocarditis, in many cases of *secondary infection* following scarlet fever, and in diphtheritic false membranes.

THE STREPTOCOCCI OF ERYSIPELAS.

The presence of streptococci in erysipelatos inflammations of the skin was first observed by Koch. Fehleisen cultivated the cocci artificially, and demonstrated their pathogenic properties. According to the above authorities they are more numerous upon the margins of the erysipelatos area, and may even be seen in the lymph channels a little beyond the red margin which marks the line of progress of the infection. This organism is considered by many to be identical with the preceding.

Microscopical Appearances.—Micrococci arranged in chains, consisting of either a few or many individual cocci.

Motility.—Non-motile.

Staining Reactions.—Any of the watery solutions of the anilin dyes can be used. It is also stained by the Gram and Claudius methods.

Biological Characters :—

On Gelatine Plates small dot-like greyish-white colonies form, which macroscopically appear opaque and coarse-grained. The colonies never attain a very great circumference.

In Gelatine Stab-Cultures small round white colonies form along the track of the needle.

On Agar Plates kept at 37° C. small colonies develop which do not attain any great size.

In Stroke-Cultures on Nutrient Gelatine or Agar small discrete round transparent colonies like dew-drops develop along the inoculated part.

In Bouillon the growth is better than in the solid media, a ropy sediment being formed, which, when the tube is shaken, rises in the fluid. Microscopically examined, the sediment is found to consist of long chains of cocci. (See Photomicrograph, Fig. 35.)

On Potatoes they grow poorly or not at all.

Pathogenesis.—Rabbits subcutaneously inoculated in the ear exhibit an erysipelatos inflammation extending from the point of inoculation to the head and neck. The temperature rises, and reaches its maximum in from eight to ten days. The animals recover.

Fehleisen inoculated cultures obtained from the skin of patients with erysipelas into patients suffering from lupus and carcinoma, and

obtained positive results, a typical erysipelatous inflammation having developed around the point of inoculation, after a period of incubation of from fifteen to sixty hours. This was accompanied by chilly sensations, and an elevation of temperature. Persons who had recently recovered from an attack of erysipelas proved to be immune.

STREPTOCOCCUS PERNICIOSUS PSITTACORUM.

This organism was found by Eberth and Wolff in parrots imported into Europe, the mortality being very great. Nodules were present on the surface of the lungs, spleen, and kidneys. In the bloodvessels of the nodules, and in the heart's blood, medium-sized cocci were found with a tendency to form chains. It must, however, be noted that parrots often die of chicken cholera, the bacillus of which, with an inferior lens, might be mistaken for cocci. Parrots are also often affected with tuberculosis, during the course of which disease mixed infection with streptococci may occur (Hirsch and Kolle).

The disease produced by this organism is of considerable importance, as being probably the source of some obscure forms of lung infection in man, which have been traced to diseased parrots kept as pets.

DIPLOCOCCUS OF PNEUMONIA (FRÆNKEL).

This organism occurs frequently in the exudate in pneumonia and secondary affections associated with that disease (pleuritis, pericarditis, peritonitis, meningitis, endocarditis, etc.). It is usually present in the sputum of pneumonic patients, and it is also found in normal sputum of healthy individuals. It is known by a variety of names: Micrococcus or Diplococcus lanceolatus, Pneumococcus, etc.

Microscopical Appearances.—Spherical or oval cocci, usually occurring in pairs, but sometimes forming chains of three or four elements. In stained specimens from the fibrinous exudates of croupous pneumonia, and from the blood of inoculated animals, a capsule is visible surrounding the cocci. The capsule is also occasionally seen in stained preparations from the surface of cultures on blood-serum. (See Fig. 36.)

Motility.—Non-motile.

Staining Reactions.—The diplococcus stains readily with the usual aniline stains, and by the Gram method, which distinguishes it from Friedländer's bacillus of pneumonia, the latter being decolorized.

To demonstrate the capsules in cover-glass specimens, place the specimen in 1 per cent. acetic acid for one minute, dry, and stain with Ehrlich's anilin-water gentian-violet; or stain by Johne's method. (See p. 27; also Fig. 36, stained by Johne's method.)

Biological Characters.—The pneumococcus is an *aërobic*, but is also a *facultative anaërobic* organism. It retains its vitality and virulence much longer under anaërobic conditions. The minimum temperature for its growth is 22° C., maximum 39.5° C. for cultures on solid media and 42.5° C. for those in liquid media, while the optimum temperature is 35° to 37° C.

On Gelatine at 25° it develops fine delicate colonies. *The gelatine is not liquefied.*

On Oblique Surface Agar (which must be only slightly alkaline), as also on *Agar Plates* and blood-serum, the diplococci grow in small, granular dewdrop-like colonies.

In Bouillon the growth exhibits nothing characteristic.

Milk is a favourable medium, and in some cultures coagulation occurs.

The diplococci grow best on media containing blood.

Vitality.—Pneumonic sputum smeared on clothing, air-dried, and exposed to diffuse daylight, retained its virulence for rabbits in one series of experiments for a period of nineteen days, and in another series for fifty-five days. Exposed to direct sunlight, the same material retained its virulence after twelve hours' exposure. In agar cultures the diplococci do not live long (four to five days), but in bouillon their vitality is more prolonged. The apparent cause of the cultures dying is the formation of lactic and formic acids. Neutralizing the cultures with calcium carbonate causes them to retain their vitality for months. Exposure for ten minutes at 52° C. is sufficient for their destruction, and they exhibit very slight resistance to the ordinary germicides.

Pathogenesis.—The diplococci of pneumonia are pathogenic for rabbits, guinea-pigs, and mice. Rabbits inoculated subcutaneously with a fresh virulent bouillon-culture die in one to two days from typical septicæmia; rats are less susceptible to infection, and chickens and pigeons are immune. Kruse and Pansini also found a sheep and a horse immune.

MICROCOCCUS INTRACELLULARIS MENINGITIDIS.

This micro-organism, which is the cause of epidemic cerebro-spinal meningitis, was discovered by Weichselbaum in 1887. It is frequently referred to as the *Diplococcus intracellularis*, the name "intracellularis" having been given to it because of the fact that the germ is most frequently found lying within the pus-cells of the cerebro-spinal fluid and the meningeal exudation. It has also been found in nasal secretion.

Microscopical Appearances.—Micrococci, usually occurring in pairs, occasionally in tetrads, and somewhat resembling the gonococcus. Jæger observed the formation of chains of cocci, the cocci in the chain proceeding to divide along a line parallel to the length of the chain; in consequence of this, there were here and there tetrads formed along the line of the chain.

Motility.—Non-motile.

Staining Reactions.—Stains easily with the ordinary dyes, but not by the Gram method, unless the process of decolorization is brief. The latter fact explains why some authors state that the diplococcus does stain according to Gram.

Biological Characters.—On *Gelatine Plates* a slight growth is observable.

In Gelatine Stab-Cultures it first grows in the deeper layers, then in the upper, but not upon the surface. The gelatine is not liquefied.

In Bouillon a slight clouding is observable after twenty-four hours at 37° C. After a time a more and more plentiful precipitate forms, which is of a slimy character, but can, through continued shaking, be brought into uniform suspension in the fluid.

Glycerine-Agar slant-cultures at 37° C., show growth along the track of the needle after twenty-four hours, the colonies having a yellowish-white appearance when viewed by transmitted light. Stadelmann and Blumenfeld (1899) found that the growth had reached its maximum after twenty-four hours.

On Potatoes a very delicate growth is observable after forty-eight hours at 37° C., the colonies appearing as whitish dots. After some days these colonies become moist and confluent, and take on a citron-yellow colour.

In Milk the diplococci remain alive for a considerable time, without undergoing much development. The milk remains unchanged.

Pathogenesis.—Infection is supposed to occur under natural condition by the germ being inhaled into the nasal cavity, penetrating thence through the cribriform plate into the skull-cavity or beneath the brain-membranes. About 30 to 50 per cent. of the persons attacked succumb. The organism has been found in a large number of cases; pure cultures being obtained by lumbar puncture during life. The operation seems to benefit the patient, and when combined with a bacteriological examination, is a valuable aid to diagnosis. As Councilman, Mallory, and Wright (1898) have shown, a large number of cultures should be prepared from each case, as many fail to develop a growth, though diplococci may be found microscopically in smear-preparations.

Of the experimental animals, white mice were found to be the most susceptible by Weichselbaum, these animals being killed in thirty-six to forty-eight hours by intraperitoneal or intrathoracic injection of cultures. He found the dog, rabbit, and guinea-pig to be but slightly susceptible. Lesions similar to those in man have been produced by injections into the spinal canal of goats. Subcutaneous inoculations of experimental animals have given negative results.

Vitality.—The organism usually exhibits low vitality in cultures. In some cases it has, however, been successfully transplanted after as long as four weeks. Its resistance to dessication varies greatly, depending upon the source of the organism. Councilman, Mallory, and Wright obtained no growth from organisms which had been dried forty-eight hours on paper, but Jæger (1894) obtained successful cultivations from a handkerchief used six weeks previously by a patient. Finally, Germano found diplococci alive after being kept dry for over ninety days. So we may perfectly well conclude that the germ may float in the air with dust particles, and lead to infection through inhalation as indicated above. The organism is highly susceptible to disinfectants.

MICROCOCCUS MELITENSIS.

This organism was discovered by Bruce in 1887, and is recognised to be the cause of Malta or Mediterranean fever, a disease which is, however, not confined to these parts of the globe. Bruce isolated the germ from the spleen of living persons suffering from the disease, as also at autopsy.

Microscopical Appearances.—Minute round or oval bodies 0.5μ

in diameter, occasionally forming chains in cultures. In old cultures, or such as have been kept at room temperature, bacillus-like forms may be encountered (Durham), but these are doubtless due to degeneration.

Motility. — Slightly motile. Gordon has demonstrated the existence of one to four flagella.

Staining Reaction.—Readily stained with the ordinary dyes, but not by the Gram method.

Biological Characters. — The organism develops slowly in cultures kept at 20° C., the optimum temperature of growth being 37°.

In Gelatine Stab-Cultures growth proceeds slowly, and is not accompanied by liquefaction. After about four weeks a slight growth about the size of a pin-head is evident upon the surface of the medium, and a visible multiplication has taken place along the track of the needle.

In Glycerine-Agar Slant-Cultures made from spleen pulp small round, slightly raised, translucent colonies about 2 to 3 mm. across, appear about the third day in the incubator. After three weeks the colonies are no larger than a millet-seed. Viewed by reflected light the colonies are pearly white, by transmitted light they are yellowish and translucent. Where the growth is continuous immediately along the track of the needle, the edge of the growth is serrated. Old cultures assume a buffy tint.

Bouillon becomes uniformly turbid; no pellicle is formed; ultimately a flocculent precipitate forms.

Pathogenesis.—The disease has been reproduced by inoculation in monkeys. Out of seven monkeys inoculated with pure cultures by Bruce and Hughes, four died and the micrococcus was regained from the animals after death. Three animals recovered after an illness of three months, having had similar symptoms to those observed in man, in whom the disease lasts on an average about ninety days. Rabbits, guinea-pigs, and mice normally resist infection, but Durham has found that he could raise the virulence of the germ for guinea-pigs by intracerebral inoculation of these animals, the organism becoming capable of killing guinea-pigs by intraperitoneal inoculation.

The mode in which infection occurs naturally in man is unknown. The mortality is low, only about 2 per cent. of the persons attacked

succumbing. The period of incubation would appear to last from six to seventeen days. The disease is characterised by continued fever with irregular intermissions, profuse perspirations, pain in the joints which at times become swollen, orchitis, and, as a rule, constipation. The organism is especially abundant in the spleen, which is the organ of the body most affected. The spleen is greatly enlarged, the kidneys show glomerular nephritis, the other organs chiefly show a condition of cloudy swelling.

Diagnosis.—Will depend upon the character of the fever and the finding of the specific germ through splenic puncture. The absence of malarial parasites would exclude malaria. We have a specific reaction in the phenomenon of *agglutination*. The blood of persons affected and for a considerable time after recovery is capable of agglutinating the *Micrococcus melitensis*.

BACILLUS OF PNEUMONIA (FRIEDLÄNDER).

This organism is seldom found in pneumonic patients. It occurs either alone or associated with other organisms, and is frequently found in the nasal discharge in *catarrh*, and in *otitis media acuta*.

Microscopical Appearances.—The bacilli are much larger than the diplococcus of pneumonia, the minimum size being $1\ \mu$. They are arranged in pairs or in chains. A capsule is present in specimens from sputum and inoculated animals, and it can also sometimes be observed in specimens prepared from cultures. (See Photomicrograph, Fig. 37.)

Motility.—Non-motile.

Staining Reactions.—It stains easily with the ordinary anilin dyes, but not by the Gram method.

Biological Characters.—It is aërobic and facultative anaërobic, growing both at room and incubator temperatures.

On Gelatine Plates it forms small elevated porcelain-like colonies; the gelatine is not liquefied, but eventually acquires a brownish colour.

In Gelatine Stab-Cultures a typical nail-shaped growth occurs.

On Agar Media it forms a whitish coating.

On Potatoes at incubator temperature it develops a yellowish-white coating which contains gas bubbles.

Media containing grape sugar undergo fermentation, CO_2 , H_2 , alcohol, and acetic acid being formed.

Milk is not coagulated.

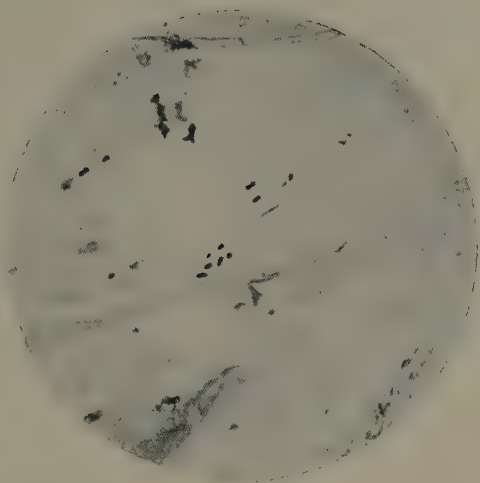


FIG. 36.—*Diplococcus pneumoniae* (Fränkel). Cover-glass specimen from pneumonic sputum, showing capsules. Johne's method. $\times 1000$.

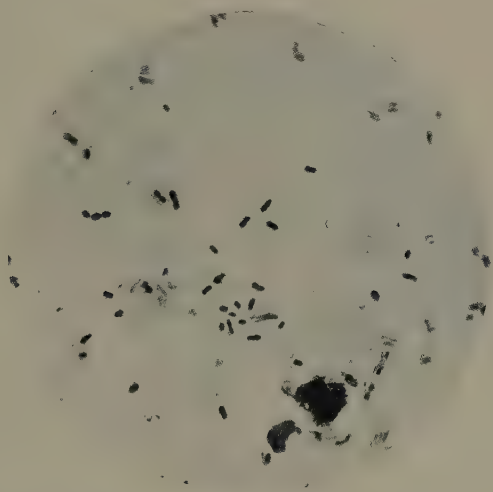


FIG. 37.—*B. pneumoniae* (Friedländer). Cover-glass specimen from inoculated mouse. Bacilli showing capsules. Johne's method. $\times 1000$.

Vitality.—At 40° C. development ceases. The thermal death-point is reached at about 56° C. The bacilli retain their vitality in ordinary culture media for a long time, living for several months.

Pathogenesis.—The bacillus of pneumonia is pathogenic for mice and dogs, and slightly so for guinea-pigs. It is distinguished from the diplococcus of pneumonia by rabbits being immune to its action. Susceptible animals may be inoculated direct into the pleural or abdominal cavities, but they may also be infected through inhalation of dry pulverised cultures. In some cases pneumonic lesions are produced.

STREPTOCOCCUS OF STRANGLES OF THE HORSE (SCHÜTZ).

Synonyms.—*Ger.* Druse der Pferde ; *Fr.* Gourme.

Strangles is an infectious catarrh of the upper air-passages of the horse, accompanied by suppurative inflammation of the neighbouring lymph-glands which generally leads to abscess formations. The disease is often complicated with metastatic abscesses in distant organs and lymph-glands, the virus being carried thither by means of the blood and lymph circulation. One attack gives immunity for years, perhaps for life.

Microscopical Appearance.—The Schütz streptococcus is found in the pus obtained from the lymph-gland abscesses. It occurs in more or less long bundles of chains. The most characteristic formation is when the cocci are arranged in threads lying either slightly bent or undulating between the pus corpuscles (see Photomicrograph, Fig. 38). Individual cocci in the chain sometimes appear larger than the others.

Staining Reactions.—The streptococci stain very well with fuchsin and gentian-violet. For pus preparations the Gram and Claudius methods give good results, decolorization being effected with a saturated alcoholic solution of fluorescein according to Kühne's modification.

Biological Characters.—*In Gelatine* the growth is weak, white colonies forming along the track of the needle. The gelatine is not liquefied.

On Agar inoculated with a drop of pus, numerous transparent colonies about the size of a pin-head develop. In stab-cultures at 37° C., they form a greyish-white zone with wing-like projections.

On Solidified Blood-Serum at 37° the growth is most luxuriant. The colonies appear at first as shiny grey drops, subsequently a dry iridescent coating forms.

In Bouillon a flocculent white mass develops, finally forming a sediment at the bottom of the tube.

Pathogenesis.—Horses inoculated with pure cultures acquire abscesses at the point of inoculation. Inoculation into the mucous membrane of the nose causes typical purulent nasal catarrh, with accompanying inflammation and enlargement of the lymph-glands. Mice are also susceptible to infection when inoculated, an abscess forming at the point of inoculation accompanied with metastatic suppuration throughout the course of the lymphatic and blood circulations.

Differential Diagnosis.—The disease is differentiated from glanders by experimentally inoculating field-mice, which are highly susceptible to glanders, but immune to strangles.

The streptococcus of strangles is not a very resistant organism, as white mice inoculated with dried pus remain unaffected.

STREPTOCOCCUS OF CONTAGIOUS MAMMITIS OF MILCH COWS.

This is a special form of mammitis occurring in milch cows, and has been described by Nocard and Mollerau. The disease passes rapidly from one animal to the other. It commences at the base of the teats in the form of indurated lumps, which sooner or later invade the whole organ.

Microscopical Appearance.—Round or ovoid micrococci, 1.25 μ long and 1 μ broad, occurring in long straight or undulating chains. These characteristic chains are found in the milk and in the walls of the excretory ducts. They can be stained by the ordinary dyes, but not by the Gram method. The growth of this organism in cultures is checked by a trace of boric acid. By means of injections of tepid 4 per cent. solutions of boric acid into the teats of affected udders, Nocard and Mollerau succeeded in arresting the extension of the disease. The organism is also destroyed by a 3 per cent. solution of carbolic acid.

Biological Characters.—*On Gelatine Plates* small, round, slow-growing non-liquefying granular colonies.

Gelatine Stab-Cultures: the growth has the form of a nail.

Agar-Agar: grows badly.

Bouillon is very soon clouded.

Milk becomes acid and is coagulated.

Pathogenesis.—Pure cultures introduced into the teats produced the disease in the cow and goats. The dog, cat, rabbit, and guinea-pig remained unaffected by both intravenous and intraperitoneal injections.

The disease is communicated from the diseased to healthy cows by the hands of the milkers, which can be prevented by disinfection of the hands with 3 per cent. solution of carbolic acid. Milk from a diseased cow also infects the milk from the healthy animals to which it may be added, rendering *both unfit for human consumption*.

STREPTOCOCCUS OF CATARRHAL MASTITIS.*

History.—Zschokke considers that this organism is the cause of infectious catarrhal mastitis.

Morphology.—Two varieties of this germ are described, a short variety forming chains consisting of eight to forty cells, and a long variety forming chains with one hundred to two hundred cells measuring $\frac{1}{2}$ to 1μ in diameter. The short variety is generally found within leucocytes, whilst the larger variety is mostly found outside of cells. The small variety also occurs free, and is generally associated with the severe acute forms of the disease.

Growth:—

In Gelatine Stab-Cultures both varieties develop brush-like colonies, the lateral branches of which have swollen ends. The medium is not liquefied. The organism develops under aërobic and anaërobic conditions, both in alkaline and neutral media. The addition of milk-sugar favours its development.

In Bouillon, milk, and in horse- or ox-urine the growth is rapid and luxuriant; there is no cloudiness, but a flaky white mass is formed. Both forms very quickly cause an acid reaction (lactic acid formation, Nencki). The long forms appear to withstand the acidity of the medium better than the short ones.

The streptococcus does not possess great resistance. Cultures die after a few hours' exposure to direct sunlight, or to a temperature of 42° C. As long as affected animals are milked daily the bacterial

* Kitt, *Bakterienkunde und pathologische Mikroskopie*, Wien, 1899.

development is encouraged and the disease may continue for months. The long variety remains living a long time in the udder of dry cows, and resists acids and phagocytosis more than the short variety, whereby the mastitis assumes a chronic character. Zschokke mentions a case in which the disease persisted for six months.

Animals affected.—Pure cultures, or milk containing the germ injected into the udder or teats of cows or goats, produced the typical lesions of the disease.

Pathology.—The milk in cases of streptococcus mastitis always contains pus cells and often red blood corpuscles. The principal structural changes are cellular infiltration, purulent catarrh, and atrophy. The alveoli are filled with epithelial cells and leucocytes. Chains of cocci are found in the leucocytes, milk-teats, epithelial spaces, and connective tissue of the gland.

MICROCOCCUS TETRAGENUS.

This micrococcus was discovered by Koch in 1884 in a phthisical lung cavity. Gaffky described its pathogenic effects on various animals. Biondi also found it in human saliva.

Microscopical Appearances.—When obtained from the animal body it occurs mostly in groups of four surrounded by a capsule.

Motility.—Non-motile.

Staining Reactions.—It stains with ordinary dyes and by the Gram method, the protoplasm remaining stained while the capsule is decolorized.

Biological Characters.—It grows at 20° C., best in the presence of oxygen on the usual media at from 35° to 38° C.

On Gelatine Plates white, shiny, prominent round colonies develop.

In Gelatine Stab-Cultures it grows on the surface as well as along the track of the needle; on the surface it forms a white, shiny, prominent growth. The gelatine is not liquefied.

On Agar it forms a white, moist, irregularly outlined growth.

On Potatoes, a shiny, thick, irregular patch is formed.

Pathogenesis.—In white mice and guinea-pigs it produces local abscess, sometimes general septicæmia. Grey mice, dogs, and rabbits are not susceptible. Macroscopically, no alteration can be observed in

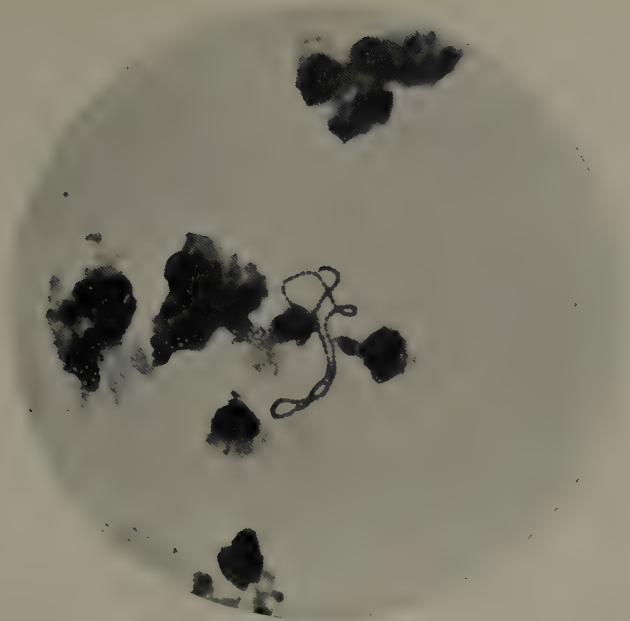


FIG. 38. Schütz's Streptococcus of strangles in pus from abscess. Claudins stain. $\times 1000$.

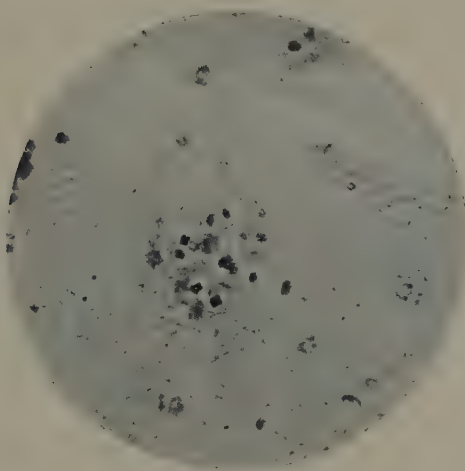


FIG. 39.—*M. tetragenus* in section of spleen of inoculated mouse. Gram-Günther method. $\times 500$.

the organs of dead animals, but microscopical examination will reveal the presence of the organism. In sections in cases of septicæmia the organisms will always be found within the capillaries. (See Photomicrograph, Fig. 39.)

MICROCOCCUS GONORRHEÆ.

This organism was discovered by Neisser in 1879 in gonorrhœal pus, and described by him as the gonococcus. It was cultivated by Bumm in 1885, and its specific pathogenicity proved by inoculation into men. It is constantly present in virulent gonorrhœal discharges, generally in the interior of the pus cells, or attached to the surface of the epithelial cells.

Microscopical Appearances.—Cocci usually occurring in pairs, in which case they are shaped like a coffee bean, or like a pair of kidneys placed with the hila in apposition, and are separated by a distinct interspace. The length of the gonococci is $0.8\ \mu$ to $1.6\ \mu$, diameter 0.6 to $0.8\ \mu$.

Staining Reactions.—It stains quickly with methyl-violet, gentian-violet, fuchsin, not so quickly with methylene-blue, which is, however, the best stain for demonstrating its presence in pus. (For special staining methods, see p. 24.) The results with the Gram method are negative, which enables it to be distinguished from other common pus cocci. Though, according to Bumm, other diplococci sometimes occur in gonorrhœal pus that do not stain by the Gram method, the most trustworthy diagnostic character is that the gonococci are found within the pus cells sometimes in one or two pairs only, frequently in considerable numbers, and sometimes almost filling the cells. (See Photomicrograph, Fig. 40.)

Biological Characters.—The gonococcus grows only at 37°C .

Plate-Culture Method.—The gonorrhœal discharge is inoculated into a tube containing fluid human blood-serum, and two dilutions are made in the usual manner into two other blood-serum tubes, all three being maintained at 40°C . In these three tubes an equal quantity of 2 per cent. peptone agar, previously dissolved and cooled down to 40°C ., is added, and three plate-cultures in Petri-dishes are prepared and placed immediately in the incubator. In twenty-four hours isolated colonies of the gonococcus appear. The superficial colonies exhibit a dark spot in the centre, from which a delicate, finely granular coating extends round about the colony; the deeper colonies

are greyish-white in colour, and possess an uneven appearance, and in two to three days acquire the shape of a blackberry. In re-inoculating from the colonies they are found to consist of a shiny tenacious compact mass.

*Stroke-Cultures on oblique solidified Blood-Serum-Agar are prepared as follows:—*1 part of fluid human blood-serum at 40° C. is mixed with 3 parts of melted agar, also at 40° C., and placed in an oblique position to solidify. The growth on this medium is luxuriant; at first isolated grey colonies appear, which later become moist, shiny, tenacious, and slimy, and from the margins a thin film-like coating extends.

A good liquid medium is prepared by mixing 1 part of human blood-serum with 2 parts of peptone bouillon. In this medium the gonococcus forms a membrane on the surface, while the medium itself remains almost entirely clear.

In preparing the culture media animal blood-serum can be used instead of human serum, although they do not grow so well, nevertheless gonococci grow quite well on swine blood-serum.

Pathogenesis.—Bumm made inoculations into the healthy urethra in two cases, once with a third generation culture and once with a culture of the twentieth generation. In both cases a typical gonorrhœa developed as the result of the inoculation. The mucous membranes of man most liable to gonorrhœal infection are those of the urethra, conjunctiva, the cervix uteri, and in children that of the vagina. Inoculations of gonorrhœal pus into the vagina or conjunctival sac of the lower animals, dogs, rabbits, horses, apes, are without result.

In *Blenorrhœa neonatorum*, according to Bumm, after infection the presence of gonococci may be demonstrated in and between the superficial epithelial cells of the mucous membrane; they soon penetrate to the deeper layers, and by the end of forty-eight hours the entire epithelial layer is invaded by the cocci. They also multiply in the superficial layers of the connective tissue, and give rise to inflammatory reaction, which is shown by an abundant escape of leucocytes from the capillary network.

Bacteriological Diagnosis.—The microscopical examination of the urethral discharge is of the greatest importance. Cover-glass specimens are prepared from suspected discharge, air-dried, fixed in the flame, and stained with a watery solution of methylene-blue.

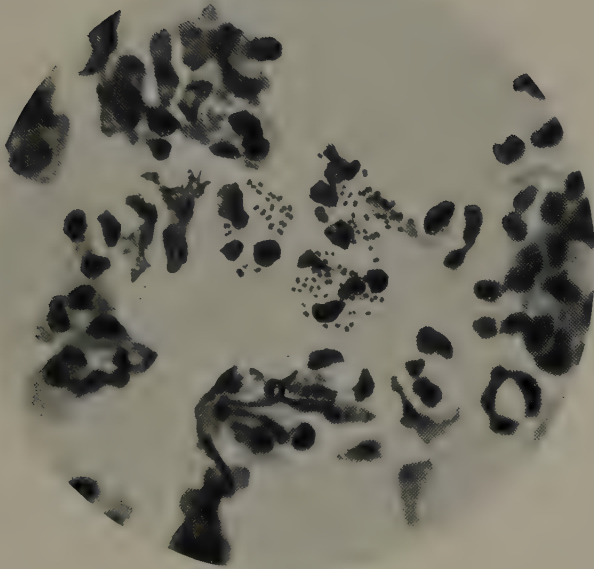


FIG. 40. *Micrococcus gonorrhoe* in urethral discharge. Stained by Knack's method. $\times 1000$.

T. Bowhill, F.R.C.V.S., Photo.]

[Face page 124.

The cocci and nuclei of the pus-cells are stained blue by this method, the cocci more intensely than the nuclei. The characteristic form of the cocci, their position in the pus-cells, and their negative reaction with the Gram stain, allow the diagnosis of gonorrhœa to be made with certainty. The gonococci are sometimes situated deep in the mucosa of the urethra, for which reason they may often be absent in the superficial discharge. It is therefore necessary in doubtful cases to irritate the urethra and stimulate the discharge. If the secretion after repeated examination, also with previous irritation of urethra, is found free from gonococci (numerous other cocci are usually present), then the infection can be considered to have subsided.

BACILLUS PYOCYANEUS.

This bacillus is found in green or blue-coloured pus, especially in green-coloured bandages, from which pure cultures may be easily obtained by means of plate-cultures.

Microscopical Appearances.—Small thin bacilli (about the length of the bacillus of mouse septicæmia, but a little thicker), which in cultures often form small chains, occasionally filaments.

Motility.—Actively motile, possessing only one flagellum.

Spore Formation.—Not known to occur.

Staining Reactions.—It stains readily with the ordinary anilin dyes, but not by the Gram method.

Biological Characters.—This organism is a *facultative anaërobe*, growing both at room temperature and in the incubator.

On Gelatine Plates, flat, irregular, circumscribed colonies develop with radiating borders; the gelatine is quickly liquefied, the surrounding medium exhibiting a green fluorescence.

In Gelatine Stab-Cultures the medium is quickly liquefied.

On Oblique Surface Agar a whitish coating is formed, and the underlying medium is coloured green.

On Glycerine Agar the inoculated medium exhibits at first a blue colour, which gradually becomes darker.

On Potatoes a greenish-yellow or brownish growth takes place, the surrounding surface being coloured green.

Bouillon is clouded.

Milk is coagulated and peptonised.

This organism produces pyocyanin and a fluorescent green colour-

ing matter, formed only in the presence of oxygen. This pigment is soluble in chloroform.

Pathogenesis.—This bacillus is pathogenic for guinea-pigs and rabbits, 1 c.c. of a bouillon culture causing the death of the animal in from twelve to thirty-six hours. Smaller amounts do not kill the animals, but render them immune to doses fatal to animals not previously protected. In rabbits inoculated with a culture of the anthrax bacillus a fatal result may be prevented by inoculating the animal with a pure culture of the bacillus pyocyaneus soon afterwards. Woodhead's experiments indicate that the antidotal effect is due to the chemical products of the growth of the bacillus and not to an antagonism of the living bacterial cells.

MICROCOCCUS OF GANGRENOUS MAMMITIS OF MILCH EWES.

Gangrenous mammitis, also called *mal de pis araignée*, causes great mortality in the affected ewes.

Microscopical Appearance.—According to Nocard, the specific germ is a very fine micrococcus 0.2μ in diameter, occurring in groups of four or more, never in chains.

Staining Reactions.—Stains by the Gram method.

Biological Characters.—It is a facultative anaërobe.

On Gelatine Plates. Grows on the surface in round, white, liquefying colonies.

In Gelatine Stab-Cultures a funnel-shaped growth occurs.

On Agar an abundant yellowish-white growth develops.

Blood-serum is liquefied.

Milk becomes acid and is coagulated in twenty-four hours.

Cultures do not remain virulent unless transplanted daily.

Pathogenesis.—Cultures inoculated into the teats of a ewe produce a rapidly fatal mammitis. The goat is refractory.

Inoculations into the ordinary experimental animals cause only slight oedema, but in the rabbit an abscess forms, from which the animal, however, recovers.

DIPLOCOCCUS OF PLEURO-PNEUMONIA CONTAGIOSA OF THE HORSE.

Ger. Brustseuche der Pferde.

The Schütz bacterium is a small, slightly ovoid organism, sometimes possessing a capsule. It is found in the pulmonary tissue and

exudative pleuritis generally present in contagious pleuro-pneumonia of the horse.

Staining Reactions.—The organism stains easily with gentian-violet or methylene-blue. The Gram stain only gives positive results when the decolorization in absolute alcohol is not continued for more than fifteen to twenty seconds; after two minutes the organism is entirely decolorized. The above reaction with the Gram method differentiates this organism from Fränkel's pneumococcus, which stains readily by the Gram method.

Biological Characters.—The diplococcus grows on gelatine and agar-media at room temperature; the gelatine is not liquefied, and the growth in both media exhibits nothing characteristic.

Pathogenesis.—Affects mice, guinea-pigs, pigeons, and rabbits, but not fowls or swine. The etiological importance of this organism is established by its constant presence in the pulmonary tissues and effusions of affected horses, and by the fact that Schütz produced typical cases of the disease by injecting healthy horses intrapulmonally with the diplococcus. The germs, according to von Rust, are present in the nasal discharges of affected horses. Tiedler isolated the diplococcus from the blood of affected animals, and produced the disease in healthy animals by intrapulmonary injection.

BOTRIOMYCES.

Botryomyces (Bollinger); *Discomyces* (Rivolta); *Botryococcus ascoformans* (Kitt); *Mykodermoid* (Johns).

This organism is found in the indurated tissue of the scirrhus cord of castrated horses, and also in some forms of fistulous withers, tumours at the point of the shoulder, indurations of the skin and subcutaneous tissue, also in the connective tissue of the pelvic cavity. It has moreover been found in the pus of chronic mammitis of the cow, in the lungs, ribs, pleural cavity, and spermatic cord of swine. Four cases have been recorded in man. Macroscopically the parasite appears in the form of grape or mulberry-shaped masses of a pale greyish yellow colour and about the size of small grains of sand.

Microscopical Appearances.—The individual colonies are formed of granular clusters of germs, united by a gelatinous substance, and enveloped within a close-fitting transparent colourless membrane.

Staining Reactions.—The germ stains by the Gram method, and when eosin is used for contrast, it stains the gelatinous substance. Picric acid has a similar action.

Biological Characters.—When grown on gelatine and potato media, the conglomerations and capsules disappear. Gelatine is slowly liquefied; the developing colonies at first present a grey colour, which afterwards turns to yellow. (Kitt considers the *Botryomyces* to be a variety of the *Staphylococcus pyogenes aureus*, which seems very unlikely.)

In Gelatine Stab-Cultures it forms greyish-white threads, with slow liquefaction commencing at the periphery.

On Potatoes it forms a flat yellowish coating.

Pathogenesis.—Rabe and Kitt inoculated horses with pure cultures, and in four to six weeks genuine fibromas appeared. Mice are immune. Sheep and goats exhibit inflammation of the skin; later, necrosis accompanied with œdema. Guinea-pigs die from septicæmia. The germ generally remains localized but it may gain access to the lymph glands and blood, in which case metastatic foci may appear, these being usually localized in the lungs, skin, etc.

BACILLUS OF CONTAGIOUS ACNE OF THE HORSE.

Contagious acne of the horse is very readily transmitted to other animals. In severe cases ulcerations and inflammation of the lymphatic vessels and glands occur. It is easily distinguished from farcy by the cicatrization of the ulcers, and, moreover, the pustules do not occur in farcy. It is distinguished from horse-pox or variola of the horse by the fact that in variola the eruptions are always localized on the lips, nostrils, and pasterns. It is further identified by the presence of the bacillus which was discovered by Dieckerhoff and Grawitz in the pustules.

Microscopical Appearances.—Very small ovoid bacilli occurring singly and also forming small chains.

Motility.—Non-motile.

Staining Reactions.—Somewhat refractory to ordinary stains, but stains by the Gram method.

Spore Formation.—Absent.

Biological Characters.—In gelatine stab-cultures, white colonies about the size of a millet-seed develop along the course of the needle.

On Agar white colonies develop very slowly.

On Blood-Serum, especially from horses and cattle (37° C.), in twenty-four hours small white colonies develop on the surface of the medium, a granular deposit being formed in the water of condensation.

On Potatoes it exhibits no growth.

Pathogenesis.—Affects the horse, rabbit, guinea-pig, ox, sheep, dog, and mouse. The disease can be produced in horses by rubbing the acne scabs, or a pure culture of the bacillus into the skin. Guinea-pigs subjected to similar treatment succumb in twenty-four hours. Subcutaneous injection in dogs and rabbits produces toxic symptoms and death, but the bacilli do not spread over the body. Mice and field-mice are not infected by rubbing the germ upon the skin, but when a subcutaneous inoculation is made they die in one to ten days with the formation of abscesses, the bacilli being found in clusters in the organs. The natural disease is transmitted by means of grooming utensils, harness, blankets, etc., and is often localized in the regions covered by the saddle and the girth.

Tizzoni and Giovannini mention a case of contagious acne in man which proved fatal in thirteen days. They isolated a bacillus from the blood and skin morphologically and culturally resembling the bacillus of mouse septicæmia, but it was not pathogenic for mice, while it produced fatal results on rabbits and guinea-pigs, the conditions being similar to those found in the man. They considered this organism as only of secondary importance, and that the *Staphylococcus pyogenes* was the cause of the acne.

THE STREPTOTHRICES.

The organisms belonging to this class resemble in their structure at one time the *thread fungi*, and at other times the bacteria. Like the mould-fungi they form cylindrical threads which branch dichotomously, finally becoming visible to the naked eye as irregular radiating thread masses or mycelia. Single threads or fruit-hyphæ grow upwards out of the substance, free into the air, and break up into chains of round spores or conidia.

STREPTOTHRIX ACTINOMYCES BOVIS.

The true nature of *Actinomyces bovis* was first recognised by Bollinger in cattle in 1877. The disease is characterised by the formation of specific tumours tending to suppuration, the lesions being frequently located in the jaw-bones and subcutaneous connective tissue of the maxillary region, and are commonly known as wen, osteosarcoma, lumpy-jaw, etc., and when affecting the tongue, as "wooden tongue," owing to the indurated condition of that organ. It also occurs in the retropharyngeal lymph-glands.

The author recorded a case in a young cow where the affected parotid gland obliterating the bloodvessels was successfully extirpated. The disease has also been found in the liver, nasal cavities, larynx, lungs, and vertebræ. In the pig, the muscles, lungs, mammæ, and bones of the cervical and dorsal vertebræ are affected; a case has also been recorded in the dog; and cases of actinomycosis of the tongue in the horse are also mentioned. The disease occasionally occurs in man.

Section of a specific tumour reveals an abundance of granulation tissue, studded with soft nodules of varying size, containing numerous yellow or occasionally colourless granular bodies, the smallest appearing about the size of a grain of sand; the larger, due to the union of the smaller grains, are of different forms. The granular bodies vary in size from 0.1 to 1 mm. or more, being frequently cretaceous. The above-mentioned yellow granules are characteristic of an actinomycotic tumour. Ponfick transmitted the disease to other animals by means of those granules.

Microscopical Appearances.—Examined under a low power, the unstained granules appear as dark, finely granular, round or irregular balls. Under a high power, and after the specimen is stained, the mass is found to consist of a central zone of very fine ramified and intermingled filaments in a close network, with a few cocci arranged in the centre, and a peripheral zone consisting of radiating pyriform elements with large swollen or club-shaped extremities, which are either simple or branching. The branches sometimes subdivide, or are given off from either the pedicle or the club, the whole roughly resembling the capitulum of a daisy (see Photomicrograph, Fig. 41).

There are other forms of *Actinomyces* which are quite small, the club-like enlargements being absent, and Cornil and Babes describe

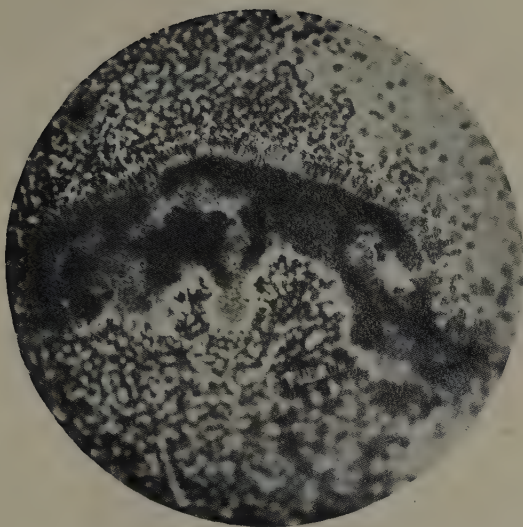


FIG. 41.—*Actinomyces bovis*. Section of a tumour from the jaw. Stained by the Gram-Günther method. $\times 450$.

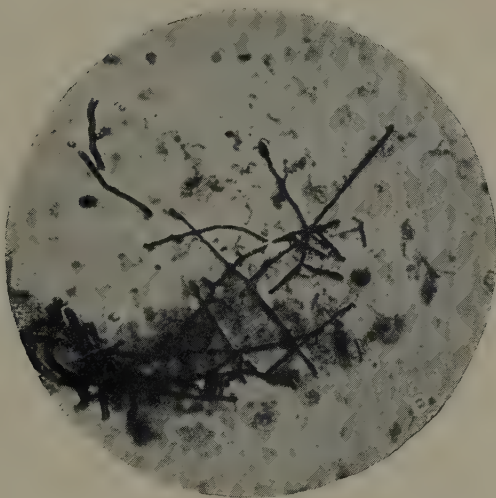


FIG. 42.—*Actinomyces bovis*. Cover-glass specimen from agar culture, showing clubs. Fuchsin. $\times 1000$.

filaments at the periphery terminating in various slight enlargements bearing conidia. The threads sometimes undergo segmentation, and resemble threads of bacilli. Finally, an agglomeration of club forms, consisting of coccus-like masses, is sometimes observed.

Unstained specimens are prepared by crushing the material under the cover-glass.

Staining Reactions.—It stains best by the Gram method, especially by Günther's modification (see Technique, p. 43), also with heated carbol-fuchsin. Double staining can be obtained with the Gram method and picro-carmin or saffranine; the threads of the fungi stain a blue-black colour by the Gram method, the clubs are stained red with carmine.

Biological Characters.—The *Actinomyces* is a typical streptothrix possessing all the characteristics peculiar to that genus. It is anaërobic and facultatively aërobic. Some authorities state that it grows best anaërobically when first obtained from the animal, whilst others consider it grows best under aërobic conditions.

On Gelatine Plates, in six days a very limited greyish-yellow growth develops, sometimes on the surface and sometimes in the depth of the medium.

In Gelatine Stab Cultures.—At the commencement a dull yellowish-white, elevated, shining, compact growth develops on the surface of the medium, sinking later from slight liquefaction of the gelatine; along the track of the needle small yellowish-white nodules develop at first, bristly outgrowths appearing later.

On Plain and Glycerine Agar opaque nodules about the size of a pin-head, which remain isolated for weeks or months, develop, their peripheries being formed of a fine delicate network. Large white nodules about the size of a lentil (rosette forms) also sometimes develop.

On slanted gelatinized Blood-Serum the individual granules exhibit a yellowish brick or red-rose colour, and they are covered with whitish downy filaments or threads.

In Bouillon the medium is not clouded, but round masses are formed in the bottom of the tube, which are separated with difficulty by shaking.

In Milk a granular growth takes place, followed by gradual peptonization.

On Potatoes the growth presents a yellowish-red colour, and is covered with a downy mass of threads.

In Eggs.—It grows well in pigeon and hen's eggs, either raw or when cooked for three or four minutes. (Prepare the egg and inoculate according to Günther's method—see Technique, p. 64). The prepared egg is placed in the incubator with the inoculated end uppermost, and examined after one to four weeks; if no putrefaction nor decolorization has taken place, the growth appears in raw eggs both in the white and in the yolk, and cloudy masses of slime resembling nasal mucus develop in the albumen. In cooked eggs opaque white spots of the size of pin-heads develop between the yolk and white, and finally a mass is formed in the track of inoculation and on the surface of the coagulated albumen.

Microscopical Examination of Cultures.—The growths on agar media consist mostly of short straight rods, but the rods are often comma-shaped, or bent. The dimensions of the rods vary; they may appear plump and thick, very slender and short, or long and thick, and sometimes club or olive-shaped at the ends (see Photomicrograph, Fig. 42). Undulating or spiral rods are sometimes observed, but these are rare in agar cultures.

In Egg Cultures beautiful net-like masses of threads occur; the threads at the periphery of the mass radiating, the ends being sometimes swollen and club-shaped.

The threads and also the short rods stain both by the Gram method and with fuchsin. When the threads are stained one hour in watery fuchsin, segments are sometimes observed, consisting of long and short rods, and short coccoid bodies arranged in an irregular manner, and separated by unstained interspaces. Finally, micrococcus-like clusters of various sizes are also found in agar and egg-cultures. They are sometimes clubbed or oval, sometimes more irregular and angular, staining intensely with gentian-violet and by the Gram method. Inoculated into fresh media they give rise to fresh rods and threads.

Vitality.—The *Actinomyces* remains living in cultures from nine to twelve months. It is killed by heating five minutes at 75° C.

Pathogenesis.—Johns transmitted the disease to the cow and the calf by subcutaneous, intraperitoneal, and mammary inoculation. Ponfick and Israel also transmitted it to the calf and to the rabbit. Israel made his inoculation with the *Actinomyces* derived from a case

in man, the results being the same as those obtained with tumours from cattle. Accidental infection is also recorded in persons attending affected animals.

The disease is supposed to be communicated to susceptible animals (Omnivora and Herbivora) by means of their food, especially straw and barley husks. Johnne found an identical fungus on the surface of husks of barley, which had become lodged on the tonsils of a healthy pig. Piana also discovered vegetable débris accompanying the *Actinomyces* in a tumour in the tongue. In a case of abdominal actinomycosis in man, Animentorp, in opening one of the abscesses, found in its centre a pin-sized concretion, in the middle of which an awn of barley was visible. A similar case was also observed in the Clinic at Vienna. In this instance the patient also suffered from abdominal actinomycosis, and a fæcal stone which was found in the patient contained a barley awn in its centre. Nocard records a case in a stableman kicked by a horse where a phlegmon developed in the upper part of the thigh, in which microscopic examination revealed the characteristic tufts of *Actinomyces*. The *Actinomyces* affecting man is distinguished from that of cattle by its tendency to form tumours, and the slow manner in which the disease spreads in the surrounding structures. The newly formed granulation tissue degenerates more quickly; suppuration is more pronounced, being accompanied by the formation of fistulæ which undermine the skin, and pass through the muscular tissue, the fungus thus spreading from the jaws or neck to the pleura and lungs, and through the diaphragm into the peritoneal cavity.

ACTINOMYCES MUSCULORUM SUIS.

This organism was discovered by Düncker in 1884, in the muscles of swine. Although resembling the *Actinomyces bovis*, it is not identical (Günther).

STREPTOTHRIX HOFFMANI.

This is another organism very similar in its growth to the *Actinomyces bovis*. It is found in the air.

Microscopical Appearances.—It occurs as a branched mycelium, with swollen, club-like ends.

Staining Reactions.—It stains by the Gram method.

Biological Characters.—It is aërobic, growing only at a temperature of over 22° C.

On Agar Media it grows in light brown warty colonies, which very soon coalesce.

Bouillon remains clear ; a thick sediment is formed.

Potatoes.—There is no growth.

Pathogenesis.—Inoculation in guinea-pigs and rabbits by subcutaneous injections produces local abscess. In the pus actinomycetes-like tufts with club-shaped ends are sometimes present, these being distinguished from *Actinomyces bovis* by the clubs staining in the same manner as the threads.

STREPTOTHRIX ACTINOMYCES HOMINIS (ISRAËL).

This organism was discovered by Israel and Wolff in two cases of human actinomycosis, and in its macroscopical, microscopical, and staining reactions resembles the *Actinomyces bovis*. The difference in cultures is, however, well marked, as it only grows luxuriantly under anaërobic conditions, and under aërobic slightly or not at all. The optimum temperature is 37°, and the development is very slow.

On Agar irregular opaque colonies are formed, which in a week attain the size of a pin-head, and usually coalesce. To obtain pure cultures, remove the pus under aseptic precautions, wash the small nodules or grains it contains in sterile water, and place them deep down in a tube of agar. As the growth develops, a thin film forms round the periphery, from which secondary nodules sometimes develop. The nodules sometimes attain the size of a lentil, and rosette forms appear.

Bouillon remains clear ; a white scaly sediment forms.

In Raw Eggs a cloudy slime forms.

In Cooked Eggs a greasy, granular mass.

In the cultures, usually straight, sometimes bent, rods with slightly swollen ends (somewhat like the bacillus of diphtheria) are found. Filamentous masses are present, as a rule, only in egg cultures.

Pathogenesis.—Affects guinea-pigs and rabbits by intraperitoneal inoculation ; a genuine actinomycosis resulting, with the formation of typical tumours.

The differential diagnosis between this and *Actinomyces bovis* would appear to depend solely on cultural characters.

STREPTOTHRIX MADURÆ (VINCENT).

Found in Madura-Foot Disease, an ulcerative affection of the feet, rarely of the hands, observed in the East Indies, America, Morocco, and Italy.

Microscopical Appearances.—The parasite consists of branched threads, 1 to 1.5 μ thick, which on the surface of some media present filaments growing upwards, spores being also formed in the substance of the medium.

Staining Reactions.—The threads and spores stain with the usual anilin dyes and by the Gram method.

Vitality.—The threads are destroyed by heating for three to five minutes at 60° C. ; the spores are killed by exposure for three to five minutes at 85° C.

Biological Characters.—Aërobic ; grows at ordinary temperature, but best at 37° C.

On Agar the growth is limited, while on *Glycerine-Agar* it is luxuriant.

Gelatine is not liquefied.

The developing colonies are nodular, hard, yellowish-white in colour, later becoming reddish.

There is no growth on serum or eggs.

In Bouillon the growth is limited, granules developing slowly.

On Acid Potatoes.—Warty, at first white, later red or orange coloured protuberances appear, which are studded with white threads.

Milk is slowly peptonized.

The growth is also very luxuriant on slightly acid vegetable infusions.

Pathogenesis.—Inoculation of various animals has produced only local reaction.

STREPTOTHRIX EPPINGERI.

This organism was found by Eppinger in a brain-abscess. It consists of a branched mycelium. Fruit-hyphæ and spores are only found in potato-cultures.

Staining Reactions.—It stains by the Gram method.

Biological Characters.—It is aërobic, growing best at 37° C.

On Gelatine, yellow warty colonies develop, which do not liquefy the medium.

On Glucose-Agar the growth is orange coloured.

On Potatoes it forms a yellowish-red coating.

Bouillon remains clear, but flaky lumps develop.

Pathogenesis.—Guinea-pigs and rabbits inoculated with this organism develop a pseudo-tuberculosis.

STREPTOTHRIX FARCINICA (ROSSI DORIA).

(*Fr.* "Bacille du farcin du bœuf," Nocard; *Eng.* Bovine Farcy.)

This bovine malady was formerly very prevalent in France, and exists also in Guadeloupe. The lesions are usually located in the limbs, and consist of swelling of the lymph-vessels, terminating at the corresponding lymph-glands, those most usually affected being the brachial, pectoral, and prepectoral groups, which suppurate slowly. When the abscesses are opened, the animal seems to recover, but other tumours subsequently appear, the animal pining and dying of general marasmus. The autopsy shows pseudo-tubercular lesions with purulent centres in the lungs, liver, spleen, and lymph-glands.

Microscopical Appearances.—Small, interwoven masses of threads, about $0.25\ \mu$ thick, arranged in tufts, are present in the pus from the abscesses.

Staining Reactions.—It stains by the Gram method, but is decolorized when the contact with the alcohol is prolonged; it also stains with Weigert's double stain. The spores stain with difficulty.

Biological Characters.—Aërobic, growing best between 30° to 40° C.

In Bouillon it forms a pellicle of a dull grey colour and oily appearance.

On Agar and Gelatine, small, more or less rounded opaque masses, thicker at the periphery than in the centre, develop.

In Milk it grows without changing the reaction or causing coagulation.

On Potatoes the growth is rapid.

Pathogenesis.—Pure cultures or pus injected into the peritoneal

cavity of a guinea-pig cause pseudo-tuberculosis of the peritoneum in nine to twenty days, the characteristic tufted organisms being present in the centre of the nodules. *Intravenous* inoculation causes a generalized pseudo-tuberculosis. Intravenous inoculation of cattle and sheep causes a slowly developing pseudo-tuberculosis.

Subcutaneous inoculation in refractory animals causes an abscess which heals quickly.

STREPTOTHRIX CAPRÆ.

Found by Silberschmidt * in the nodular lesions of the lung of a she-goat.

Microscopical Appearances.—In young cultures the mycelium breaks up into short beaded and short homogeneous segments ; and on the latter small club-like swellings sometimes appear at the extremity. In cultures on maltose-agar incubated for six weeks at 37° C. the mycelium is replaced by free spores, short chains of spores, and short chains of segments lying either singly or arranged in short chains.

Staining Reactions.—In young cultures the entire mycelium stains by the Gram method. Many segments in old cultures, when stained by the modified Ziehl-Neelsen method, are not decolorized by subsequent washing in alcohol for ten minutes.

Biological Characters.—Development takes place under ordinary aërobic conditions, and, according to Silberschmidt, not, or scarcely at all, in the absence of oxygen.

In Gelatine Streak-Cultures.—A slightly raised growth with a chalky efflorescent surface appears on the third day.

In Gelatine Stab-Cultures.—A small chalky knob develops at the upper end of the puncture, and a filmy growth along the inoculation track ; there is no liquefaction or coloration of the medium.

On Maltose-Agar at 37° C.—A drab-coloured, freely spreading growth occurs. According to Silberschmidt, the surface of the colonies is irregular, and after fifteen to eighteen days they acquire a light brown colour, and look as if powdered over with a white starchy layer.

On Horse Serum at 37° C.—A free growth occurs without liquefaction (resembling, according to Silberschmidt, a culture of the bacillus of human tuberculosis).

* Silberschmidt : " Sur un nouveau Streptothrix pathogène (*Streptothrix capræ*)," *Ann. de l'Institut Pasteur*, tome xiii., p. 841, 1899.

On Potato at 37° C.—A slightly raised, brown to grey coloured, finely granular growth develops, partly covered by a chalky-looking efflorescence; the potato acquires a brownish hue.

In Peptone Broth at 37° C.—Small isolated colonies and flocculent masses of coherent colonies develop, growth occurring at the surface as well as at the bottom of the broth.

In Alkaline Litmus Milk at 37° C.—A faint pinkish growth appears on the surface of the milk after twenty days' incubation; coagulation does not take place. No diastatic action is apparent in starch broth after fourteen days' incubation at 37° C.

Vitality.—Cultures containing spores resist exposure to 60° C. for thirty minutes, but are killed by an exposure to 75° C. for the same period, and by exposure to 70° C. for sixty minutes, and also by an exposure to 80° C. for twenty minutes. Cultures exposed in the laboratory to diffused light were successfully subcultured after seven months (Foulerton and Jones*).

Pathogenesis.—(Silberschmidt.)—Subcutaneous inoculation in rabbits produced a local abscess; intravenous inoculation produced nodules in various organs. The pulmonary nodules contain giant cells, and caseate rapidly. Four mice were inoculated subcutaneously; two were unaffected, and in the other two local abscesses formed, from one of which the *Streptothrix* was recovered. A guinea-pig inoculated subcutaneously died in eighteen days—numerous suppurating nodules being present in the parietal peritoneum, the mesentery, the diaphragm, liver, kidney, and spleen. Cultures were recovered from these nodules. Three guinea-pigs received intraperitoneal injections—one recovered, the other two died on the sixth and thirty-first days respectively. In these, numerous miliary nodules were found on the diaphragm, on the surface of the liver, mesentery, serous coat of the stomach, and on the testicles.

STREPTOTHRIX CUNICULI (ACTINOMYCES CUNICULI).

Found by G. Schmorl† in an epidemic disease amongst laboratory rabbits, and described as a "thread" bacterium belonging

* Foulerton and Price Jones: "Streptothrix Infections in the Lower Animals"—*Journal Comp. Pathology and Therap.*, vol. xiv., part i., p. 56.

† G. Schmorl: "Ueber ein. pathogenes Fadenbacterium"—*Zeitschrift für Thiermedizin*, 1891. Ref., *Centralblatt für Bakteriologie*, xi., p. 666; Foulerton and Price Jones, *loc. cit.*

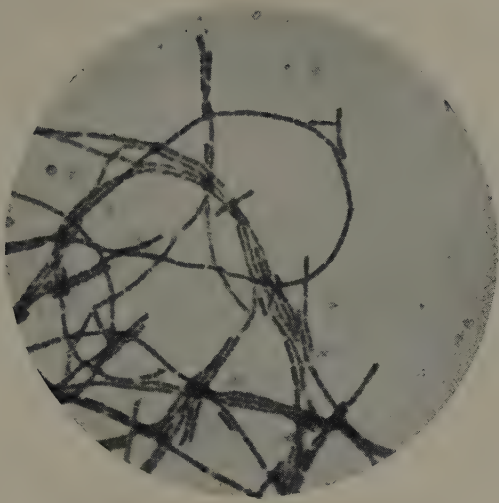


FIG. 43.—*B. anthracis*. Virulent bouillon-culture.
Methylene-blue. $\times 1000$.

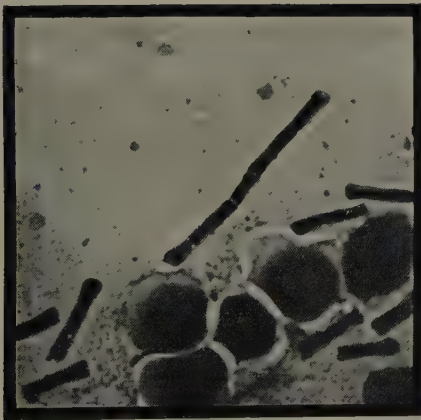


FIG. 44.—*B. anthracis*, with capsules in mouse's blood.
Stained by Johne's method. $\times 1500$.

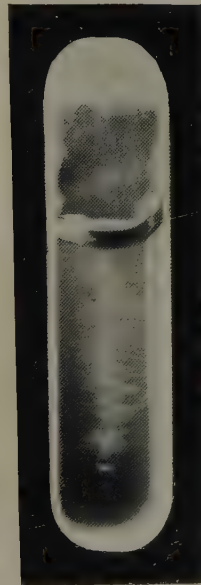


FIG. 45.—*B. anthracis*. Stab-
culture in gelatine.

to the Cladothricæ. It is an obligatory anaërobe, and cultures are obtained only on blood-serum.

Pathogenesis.—In natural infection the disease commences in the region of the mouth, and, spreading rapidly, causes necrosis of the subcutaneous tissue, associated with a fibrinous inflammation of the pleuræ, pericardium, peritoneum, and inflammatory changes in the lungs, the organism being found in the organs in every case mostly in pure cultures. Inoculation into healthy rabbits produces a similar disease, the organism being recovered from the lesions. White mice are also susceptible, but guinea-pigs, dogs, cats, pigeons, and fowls are immune.

BACILLUS ANTHRACIS.

(*Ger.* Milzbrandbacillus ; *Fr.* Bactéridie du charbon.)

This organism is always present in the blood of animals affected with anthrax, and can be isolated in pure cultures on artificial media. When susceptible animals are inoculated with portions of pure cultures, conditions similar to those found in the animal from which the original cultures were obtained are produced.

Microscopical Appearances.—In the blood of animals recently dead, the bacilli occur as large rods of variable size, from 3 to 10 μ long and 1 to 1½ μ broad, often arranged in threads formed by several conjoined rods (see Photomicrographs, Figs. 44-47). In unstained specimens examined by means of a *hanging-drop*, the ends of the rods appear round; while in stained specimens the ends of the rods are *square*. Under a high magnification the ends are found to be a trifle thicker than the body of the bacillus, and occasionally somewhat indented and concave—compared by Frænkel, when stained with methylene blue, to small pieces of bamboo cane. This latter appearance, as Johnne has shown, is due to artifice—some plasmolytic effect of certain staining reagents.

Bacilli obtained from the blood of affected animals possess *capsules* (see Photomicrographs, Figs. 44 and 46). To demonstrate the capsules, stain the specimens by Johnne's process (see p. 28). Capsules can also be demonstrated when the bacilli are cultivated in liquid blood-serum, as also at times in sections stained with methylene blue. The square ends and the presence of capsules help to distinguish the *Bacillus anthracis* from certain other organisms, especially putre-

factive bacteria. After successive passages from dog to dog, the rods become shorter and thicker (Martell).

After death, the bacilli are found in all the bloodvessels. They are easily demonstrated in sections of organs stained by the Gram or Claudius methods (see Photomicrograph, Fig. 47, showing a section of a mouse's lung with the capillaries filled with bacilli).

On artificial media the bacilli grow in long, parallel, or somewhat twisted and interlaced threads, which either form spores or degenerate, giving rise to the so-called involution forms (see Photomicrograph, Fig. 48).

Motility.—Non-motile.

Staining Reactions.—The bacilli stain easily with all the ordinary anilin dyes, with hæmatoxylin, and by the Gram and Claudius methods.

Spore Formation.—The bacilli form spores under aërobic conditions at a temperature of 15° to 37° C. Günther considers 28° C. the optimum, and that spore-formation is somewhat irregular at a higher temperature.

Spores are never formed in the living animal or in unopened carcasses. The latter is most important from a sanitary point of view, in regard to the disposal of the carcasses of animals dead from anthrax. The spores are ovoid, and one to two times as long as broad—one spore being formed in nearly every bacillus, giving the spore containing thread the appearance of a chain of beads (see Photomicrographs, Figs. 48 and 49). For the method of staining the spores of the *Bacillus anthracis*, see p. 32.

Vitality.—The resistance of anthrax spores to outside influences is not always constant. Some spores are killed by exposure to 5 per cent. carbolic acid in two days, and to steam at 100° C. in three minutes, whilst others resist 5 per cent. carbolic acid over forty days, and steam at 100° C. for more than twelve minutes. In a dry state the spores are destroyed instantly at 160° C.

When the bacilli are cultivated in bouillon to which $\frac{1}{2000}$ to $\frac{1}{5000}$ bichromate of potash is added, they lose the faculty of forming spores, and gradually lose their virulence. The power of forming spores may also be lost when the bacilli are cultivated for many generations on gelatine media.

Biological Characters.—The *Bacillus anthracis* is a facultative aërobic organism growing best in the presence of oxygen. No growth

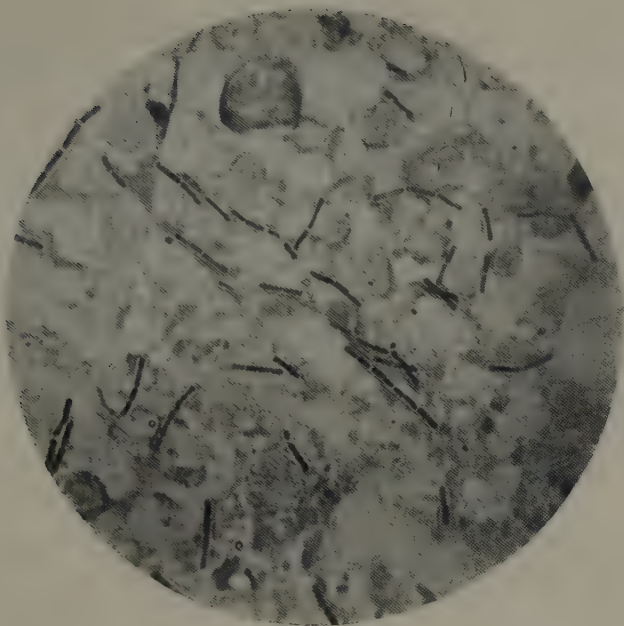


FIG. 46.—*B. anthracis* in blood of mouse. Stained by John's method.
× 1000.

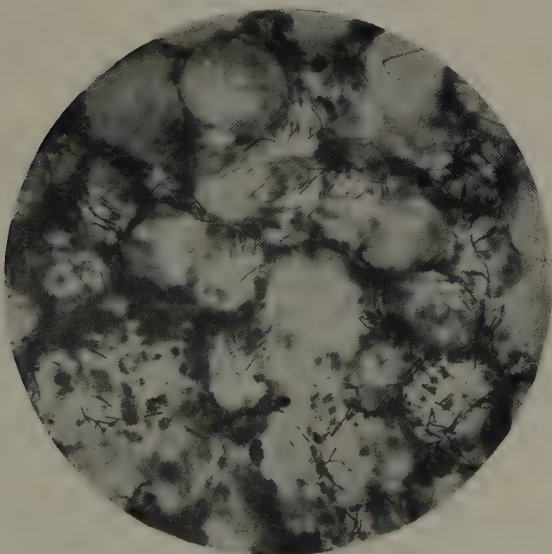


FIG. 47.—*B. anthracis*. Section of mouse's lung. Methylene-blue.
× 350.

occurs in an atmosphere of CO_2 . It grows quickly at 37°C ., but ceases to grow at a temperature under 12° or above 45°C .

On Gelatine Plates.—On the surface, small whitish colonies appear; the deeper colonies are of a greenish-black colour. Under a low power the colonies exhibit a characteristic tangled mass of single threads projecting beyond the edges of the colonies in curly hairy tufts. The colonies begin to liquefy in three to four days.

In Gelatine Slab-Cultures.—In twelve to twenty hours a thick, white growth appears along the track of the needle, from which white branching threads radiate (see Photo., Fig. 45). After two days, liquefaction commences on the surface of the medium, spreading outwards and downwards until the whole medium is eventually liquefied, and the bacterial mass sinks to the bottom of the tube.

On Agar Plates the growth is similar to that on gelatine plates, but the colonies are not so compact, and consist of masses of long threads matted together.

On slanted Agar a greyish-white tenacious coating is formed with thready edges; the condensation-water remains clear, or is only slightly clouded.

Blood-Serum is liquefied slowly.

Bouillon remains clear, but a cloudy sediment is formed.

Milk is coagulated and afterwards peptonised.

On Potato it develops as a dry granular whitish covering, more or less limited to the point of inoculation.

Pathogenesis.—Affects man, cattle, sheep, horses, guinea-pigs, rabbits, mice, and swine, the primary localization usually being in the throat. It is stated by some authors that young dogs succumb quickly to anthrax when the virus is injected into the pleura. The resistance offered by adult dogs is not absolute, as many cases are recorded of infection following the consumption of raw anthracic flesh.* Martel succeeded in exalting the virulence of the anthrax bacillus for the canine species by inoculating from dog to dog. The natural resistance of the dogs used in these experiments being enfeebled by the subcutaneous injection of phlorizidin in alkaline solution, or of pyrogallol, twenty-four hours before inoculation with the anthrax virus. He had greater success by using rabid dogs for his experiments, a single passage through a rabid

* Martel, *Annales de l'Institut Pasteur*, tome xiv., p. 13; Ref., *Journal Comp. Path. and Therap.*, vol. xiii., part i., p. 82.

dog being found to increase the virulence to such an extent that inoculation from it to other dogs proved fatal in over 70 per cent. of the cases. The sensibility of the exalted virus was found to vary with the breed, while better kept dogs were to a certain extent more susceptible than the dogs of the street. Pigeons are also susceptible to the virus exalted by passage through the dog, and the cat is very susceptible. Rats are also difficult to infect. In man the disease is named according to the manner of infection :—

Pustula Maligna.—This is the local or most common form, and results from infection through a cutaneous wound. It frequently ends in recovery, but may lead to a fatal general infection or septicæmia.

Pulmonary form.—Woolsorters' and ragpickers' disease results from the inhalation of dust charged with anthrax spores.

Bowel or Intestinal Anthrax.—Due to the consumption of anthracic meat.

Inoculation into Animals.—A minute quantity of a pure culture of the *Bacillus anthracis*, subcutaneously inoculated into mice, guinea-pigs, or rabbits, usually causes death within twenty-four to forty-eight hours. Little or no change may be observed at the point of inoculation, but the subcutaneous tissue will be usually found œdematous about the seat of inoculation. Small scattered ecchymoses occur throughout the œdematous area, the underlying muscles being pale in colour. The internal viscera show no marked macroscopical changes, except the spleen, which is enlarged, dark-coloured, and soft. The liver may present the appearance of cloudy swelling. The lungs are red or pale red in colour, whilst the heart is usually filled with blood. The disease is a true septicæmia, and after death the capillaries throughout the body always contain the typical rod-shaped organisms.

Protective Inoculation against anthrax is practised in animals, according to Pasteur's method, with two vaccines prepared from virulent cultures attenuated by cultivation at high temperature.

Vaccine No. I. is grown fifteen to twenty days at 42° to 43° C.

„ No. II. „ ten to twelve „ „

Vaccine No. I. should be sufficiently attenuated so as only to kill mice, whilst vaccine No. II. should kill both mice and guinea-pigs, but not rabbits.

Cattle and sheep receive an injection of 0.33 c.c. of a four days'

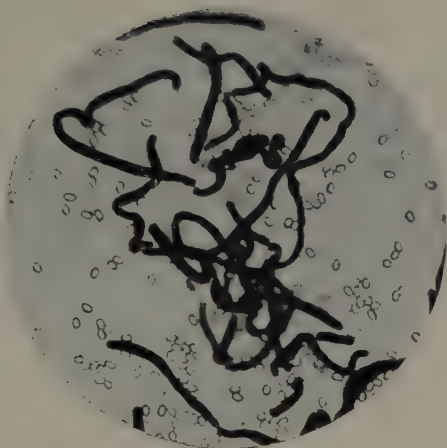


FIG. 48.—*B. anthracis*, showing commencing involution forms and free spores. Fuchsin. $\times 1000$.

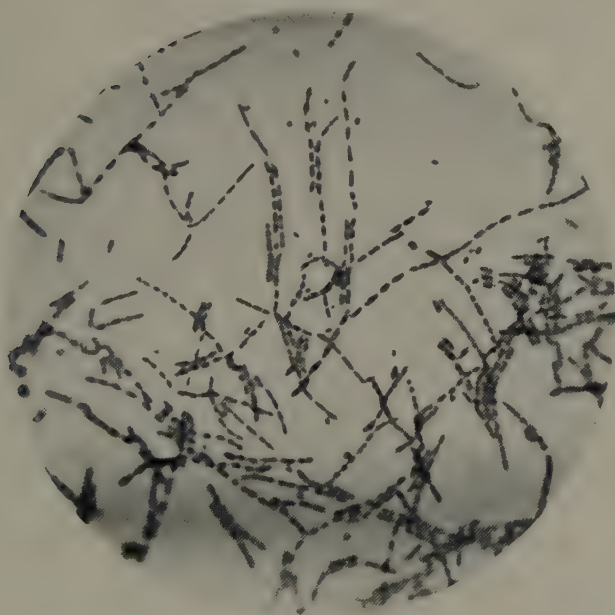


FIG. 49.—*B. anthracis*, containing spores. Agar culture. Stained with fuchsin. $\times 1000$.

old bouillon-culture of No. I., and the same dose of vaccine No. II. in ten or twelve days. The effects of the inoculation with vaccine No. I. should be scarcely noticeable, causing neither constitutional nor local symptoms. Vaccine No. II. may or may not cause constitutional disturbance, and when it does the symptoms are rarely severe, and disappear in a short time if the vaccines have been properly prepared and tested before use.

The above vaccines render sheep and cattle immune to inoculated anthrax, but, according to Koch, do not protect against natural infection by means of the intestinal tract. Pasteur's vaccines cannot be employed with certainty—the results in practice showing that the strength of the vaccines cannot be regulated with absolute certainty.

The part played by insects in conveying anthrax has recently been investigated by Nuttall. He shows that bed-bugs and fleas may be gorged on anthrax victims, and then placed on sound animals, which, on being bitten, show no disease, while at the same time cultivation and inoculation experiments made from the above insects, as well as microscopical examination, reveal the fact that the *B. anthracis* is digested in the body of the insect. He states that it is conceivable and probable that infection may occur through violently crushing the insects containing the germ against the skin, which may, moreover, be abraded through scratching.

Bacteriological Diagnosis.—Remove some of the effusion from the deeper portions of the pustule, and prepare plate-cultures. If typical colonies develop, pure cultures are made, and animals inoculated. In cases of suspected *abdominal anthrax*, the fæces and vomit must be examined. In cases of *lung anthrax*, the bacillus is sometimes found in the copious expectoration. Examination of the blood reveals whether general infection exists or not, and is of great account regarding the prognosis of the case, but it must also be remembered that the bacilli are principally localized in the capillaries.

In animals it is often necessary to decide if they have died of anthrax or not. If shortly after death a microscopical examination is made of the blood from a foot, ear, tail, or the spleen (but it is considered advisable never to cut open a suspected anthrax carcass, as it only favours the development of spores, which are never formed in the living animals or unopened carcasses), and bacilli are detected exhibiting capsules when stained by John's method (see Technique, p. 28), then the diagnosis is assured. When an animal

has been dead for several days, bacilli appear in the cadaver which are somewhat difficult to differentiate from the *Bacillus anthracis*. In such cases gelatine plate-cultures must be made, and mice and guinea-pigs inoculated. The bacillus of malignant œdema may be present in cases of mixed infection, the inoculated animal dying of malignant œdema in spite of the presence of the *Bacillus anthracis*. For this reason experimental animals should be inoculated cutaneously with the suspected material. With advancing putrefaction in a carcase, the anthrax bacilli are sometimes completely annihilated through the concurrence of other species, so that a diagnosis is no longer possible.

BACILLUS ŒDEMATIS MALIGNI.

(Fr. *Vibrion Septique*.)

This organism is very widely distributed in nature ; it is found in manured garden-earth, in filth and dust, in house drains, and also in the intestines of animals.

Microscopical Appearances.—The bacillus is somewhat narrower than the *B. anthracis*, and about the same length, but *differs in the ends of the rods being rounded*, and not squarely cut across.

Motility.—Slightly motile, possessing 3 to 12 flagella, which are attached at the ends as well as the sides of the rods (see Photomicrograph, Fig. 50. For staining flagella, see p. 29).

Staining Reactions.—The bacilli obtained from animals and young cultures stain easily with the ordinary anilin dyes.

By the Gram method positive results are only obtained when the specimen is stained for twenty-four hours at 37°, or with equal parts of anilin-water, alcohol, and 5 per cent. carbolic acid solution of gentian-violet for fifteen minutes. With the Claudius method the bacilli are easily stained in a few minutes.

Spore-Formation.—Spores are formed generally in the middle of the rods, and are sometimes broader than the bacillus, which acquires the form of a spindle or clostridium.

Biological Characters.—The bacillus is an obligatory anaërobe, growing in ordinary media both at room-temperature and in the incubator.

On Gelatine Plates the growth is similar to that of *B. subtilis*.

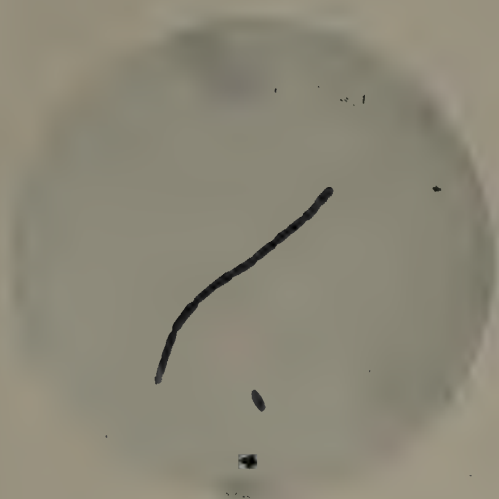


FIG. 50.—*B. edematis maligni* in mesentery of mouse.
Stained with fuchsin. $\times 1000$.

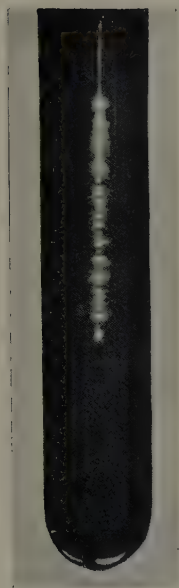


FIG. 51.—*B. edematis maligni*.
Stab-culture in gelatine.

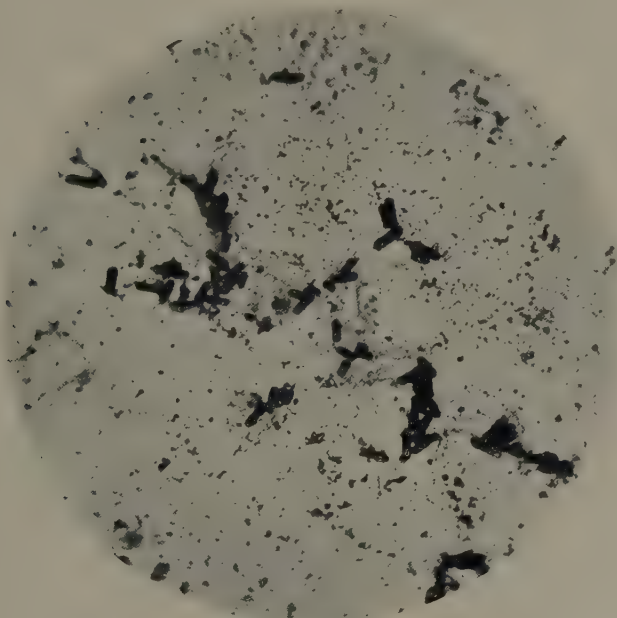


FIG. 52.—*B. anthracis symptomatici*, showing flagella. Grape-sugar-agar
culture. Stained by Bowhill's method. $\times 1000$.

On Agar Plates it forms a thick network of threads.

In Stab-Cultures in grape-sugar gelatine, the growth begins about $\frac{1}{2}$ to $\frac{3}{4}$ of an inch below the surface of the medium, in the form of a white strip, with side branches sprouting out (see Photograph, Fig. 51).

Agar Stab-Cultures, nothing characteristic.

Bouillon becomes cloudy ; gas-formation.

Milk, a part of the casein is precipitated.

Growing cultures have a most unpleasant smell.

Pathogenesis.—Susceptible are guinea-pigs, rabbits, and mice, goats, calves, sheep, horses, swine, cats, dogs, chickens, pigeons, and ducks, whilst cattle are immune. The virulence of pure cultures, when inoculated into susceptible animals, is very variable. When a small pocket is made in the skin of a mouse, and as much earth (containing the bacillus) as will cover the point of a knife introduced, the animal will die in one to two days. A general subcutaneous œdema follows, the œdematous tissue containing large numbers of the bacilli. The bacilli do not pass into the internal organs unless the animal is left lying some time after it is dead.

Several cases of malignant œdema are recorded in man. Koch mentions a case of mixed infection of malignant œdema and anthrax in a guinea-pig.

It is stated that the virus is attenuated when passed through the body of a white rat.

Immunity.—Chamberlund and Roux rendered guinea-pigs immune by injecting intraperitoneally bouillon cultures which had been sterilized in an autoclave ten minutes at 105° to 110° C.

BACILLUS ANTHRACIS SYMPTOMATICI.

(*Ger.* Rauschbrand ; *Fr.* Charbon Symptomatique ; *Eng.*

Symptomatic Anthrax, Black-quarter, etc.)

This organism was first discovered by Bollinger in cattle affected with symptomatic anthrax, and further studied by Arloing, Cornevin, Thomas, and Kitasato.

The characteristic lesions produced by this organism are emphysematous swellings of the muscular and subcutaneous tissues of the leg and the quarter, accompanied by gas-formation and strong odour.

The gases formed according to the analysis given by Kitt are: CO₂ (13 per cent.), H. (76 per cent.), N. (10 per cent.). On section of the affected parts, the muscles and cellular tissue are found saturated with bloody serum, the muscular tissue being dark, almost black, in colour. The bacillus can always be detected in the affected areas, in the gall, and, after death, in the internal organs. It has also been found in the soil in some localities.

Microscopical Appearances.—The bacilli are actively motile rods, 3 to 5 μ long and 0.5 to 0.6 μ thick, with rounded ends, usually occurring singly, but sometimes forming short threads. The flagella are attached around the periphery of the organism (see Fig. 52). The bacillus forms spores, situated either in the middle or at the end of the rods (see Fig. 54). The bacillus commences to grow at 16° to 18° C., but growth and spore-formation take place best at 37° C. During spore-formation the bacillus becomes motionless, being only motile during the vegetative stage. The bacillus also undergoes degenerative changes, and involution forms are often present, not only in cultures, but in the tissues of affected animals.

Staining Reactions.—The bacillus stains with the ordinary anilin dyes, and by the Gram method only when the staining process is prolonged. It stains easily by the Claudius method. The spores can also be stained by the ordinary methods (see p. 32).

Biological Characters.—The bacillus is strictly anaërobic, growing best in an atmosphere of hydrogen, but not in carbon dioxide.

On Gelatine Plates in an atmosphere of hydrogen the colonies appear as irregular, slightly lobulated masses. The gelatine liquefies in a short time, the colony then presenting a dark lobulated centre surrounded by a delicate fringe-like zone.

In Gelatine Stab-Cultures a radiating cloudiness appears, which increases in size as the gelatine liquefies, until finally the growth may be said to resemble a hairy caterpillar (see Fig. 53), where the characteristics of the growth correspond to those described by Sanfelice.

In deep stab-cultures in grape-sugar agar at 37° C. the growth commences in twenty-four to forty-eight hours, and is accompanied by gas formation, the amount of gas evolved being considerably more than is produced by the bacillus of malignant œdema. The gaseous products have a putrid odour, like that of rancid butter.

Milk is coagulated by the formation of an acid.



FIG. 53.—*B. anthracis symptomatici*. Stab-culture in grape-sugar gelatine.

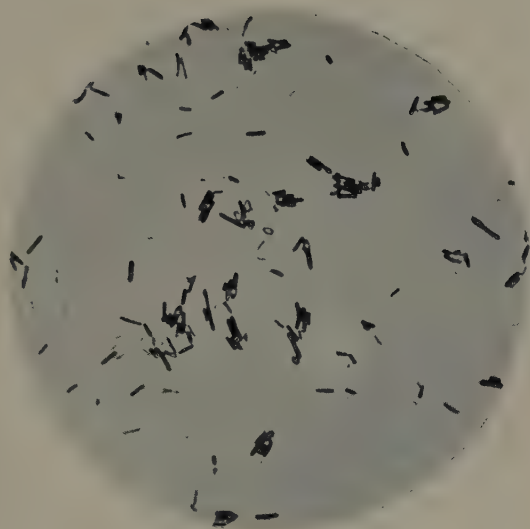


FIG. 54.—*B. anthracis symptomatici* and spores. Cover-glass specimen from grape-sugar agar culture. Claudius stain. $\times 1000$.

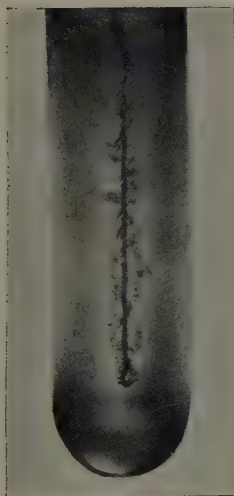


FIG. 55.—*B. tetani*. Stab-culture in grape-sugar agar.

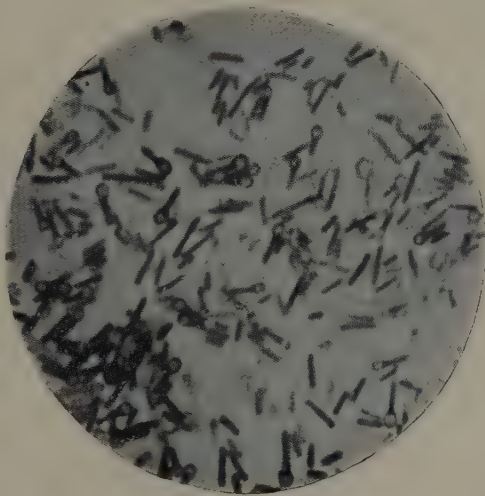


FIG. 56.—*B. tetani* and spores. Cover-glass specimen from grape-sugar-agar culture. Fuchsin. $\times 1800$.

According to recent researches by Leclainche and Vallée* the organism develops best in Martin's† bouillon sterilized by filtration, the cultures retaining their virulence much longer than those made with ordinary bouillon. These cultures also produce an active toxin, which, when introduced into the tissues by itself, produces serious effects and death in experimental animals.

The spores, in cultures that have been heated, retain the whole of their vitality, and yield very virulent cultures, although they do not germinate in the tissues. When the spore is deprived of its toxin, it is incapable of germinating and causing infection, but the addition of a certain quantity of toxin to an inoffensive dose of pure spores causes them to germinate in the tissues, and produce a typical infection.

Differential Diagnosis.—This organism is distinguished from the bacillus of malignant œdema as follows:—It is smaller, and does not develop in long threads in animal tissues; it is more actively motile, and forms spores more readily in the living tissues than does the bacillus of malignant œdema. It also differs in its reaction towards animals. Cattle are susceptible to symptomatic anthrax, but practically immune to malignant œdema. Swine, dogs, rabbits, pigeons, and chickens are readily affected by malignant œdema, but not, as a rule, by symptomatic anthrax. According to Arloing, frogs can be infected if kept at a temperature of 22° C. Horses are affected only locally and not seriously with symptomatic anthrax, but are conspicuously susceptible to both artificial inoculation and natural infection by the bacillus of malignant œdema. The distribution of the organisms over the earth's surface is also quite different, malignant œdema bacilli being present in almost all soils, whereas those of symptomatic anthrax appear confined to certain localities, especially places where infected herds have been pastured. Natural infection occurs principally in young cattle; next in order being sheep and goats; whilst a genuine case is not recorded as occurring in man.

The ordinary manner of infection is by wounds which not only tear the skin, but penetrate the subcutaneous tissue. The disease is also produced by the ingestion of forage soiled by very virulent matter, and by the inhalation of dust charged with dried virus. Lactic

* *Annales de l'Institut Pasteur*, April 1900.

† Martin: "Production de la toxine diphtherique," *Ibid.*

acid, and also acetic acid (Stockman*), when added to attenuated virus, causes it to kill like a strong virus.

Recent experiments also show that when a heated culture is thrown on a quantity of fine, sterile sand, and the mass evaporated rapidly at 38° C., broken into small particles and introduced under the skin of guinea-pigs, black-quarter nearly always results.

According to Roger,† the association of other bacteria plays an important rôle in the pathology of black-quarter, as experimental inoculations with heated cultures mixed with *Staphylococcus pyogenes albus*, and also with a *Streptothrix*, produced black-quarter in the experimental animals.

Leclainche and Vallée conclude that the resistance offered by the animal body depends, more or less, on phagocytic action, and that everything capable of retarding or preventing phagocytosis favours infection.

Vaccination.—Animals sometimes inoculate themselves accidentally, and as small doses cause immunity—this immunity being transmitted from the mother to the foetus—the result is that part of the animals exposed to contagion may escape. The French recommend the successive employment of two vaccines prepared from material from a fresh lesion spread out thinly, and dried at 35° C. A quantity of the powdery substance is triturated in a mortar with 2 parts of water, and exposed during seven hours to 100° to 104° C. for the first, and 90° to 94° C. for the second vaccine. When the dry vaccines are taken from the oven 1 centigramme of the powder is diluted in $\frac{1}{2}$ c.c. of water for each animal. The vaccine prepared at 100° is used first, and the second in eight days. The inoculation is made subcutaneously into the tissue of the ear, or under the skin of the end of the tail, the second inoculation a little above the first. Kitt recommends a single vaccine from infected flesh heated six hours at 100° C., given in decigramme doses, injected into the subcutaneous tissue near the elbow. Animals can also be vaccinated with natural virus either in the cellular tissue or intravenously; when the latter method is adopted, care must be taken to avoid inoculation of the surrounding tissue. An attenuated virus is generally preferred for the production of immunity.

* Stockman, *Veterinary Record*, No. 632, March 31, 1900.

† Roger: *Comptes rend. de la Soc. de biologie*, 1899. Ref., *Journal Comp. Path. and Therap.*, vol. xiii., part ii., p. 93.

Kitt* obtained protective serum from a cow as follows:—The animal received seven injections, for the most part of dried muscle juice, between the 16th of December and the 13th of June; the first injections were given intravenously, and the others subcutaneously. In spite of this, 50 c.c. of this cow's serum failed to immunise a sheep. After an interval of several months the cow was again treated with large doses until it no longer reacted in the least degree, and appeared to be perfectly immune; after this 10 c.c. of the serum was sufficient to protect sheep against a fatal dose of the strong virus.

GASTROMYCOSIS OVIS (NIELSEN).†

(*Ger.* Bradsot; *Scotland*, Braxy.)

This organism was isolated in a very rapidly fatal endemic disease occurring amongst sheep in Iceland and Norway. The disease is characterised by hæmorrhagic inflammation of the abomasum and other compartments of the stomach, portions of the intestines, and throughout the body. The bacilli were found in the walls of the stomach and frequently in the internal organs three hours after death. From the description of the disease given by Nielsen, it is probably identical with "Braxy," which attacks sheep in Scotland.

Morphology.—Bacilli 2 to 6 μ long, and usually 1 μ broad, often occurring in pairs, seldom in chains. Spores are formed in dead animals—the spores being formed either in the middle or at the end of the bacillus. The organism stains by the Gram method.

Growth.—It is somewhat difficult to obtain pure cultures of this organism. It develops only under anaërobic conditions, the cultures being very similar to that of the blackleg (Rauschbrand) bacillus. Growth is slow at room-temperature, but rapid at 37° C. On agar, gelatine-agar, blood-serum, or in bouillon, growth appears in twenty-four hours to three days, and is accompanied by the formation of large quantities of gas.

Animals Affected.—Mice or guinea-pigs inoculated subcutaneously with pure cultures, or pieces of infected organs, died in twelve to forty-eight hours, exhibiting a sero-hæmorrhagic œdema of the subcutaneous

* *Berliner Thierärztl. Wochenschrift*, March 1, 1900. Ref., *Journal Comp. Path. and Therap.*, vol. xiii., part i.

† Nielsen, *Monatshefte f. prakt. Thierheilk.*, 1896, Bd. viii. Jensen, *Deutsche Zeitschr. f. Thiermed.*, Bd. xxii.

tissue. Lambs inoculated with virulent material died in twelve to fifteen hours, exhibiting a severe hæmorrhagic emphysematous inflammation of the subcutaneous tissues and muscles, together with bloody transudation into the cavities of the body. According to Jensen, pigeons, chickens, a young pig, and a calf were killed in one to three days by subcutaneous injection, and at autopsy showed extensive sero-hæmorrhagic infiltration at the point of inoculation.

Vitality.—Spore-containing material heated in the steam sterilizer for five and a half hours, was slightly weakened but not destroyed. Guinea-pigs inoculated with this heated culture died in three days, and mice in two days, whereas sheep were but slightly affected.

BACILLUS TETANI.

(Lockjaw—*Ger.* Wundstarrkrampf ; *Fr.* Tetanos.)

This disease occurs in all the domesticated animals and in man. The horse, ox, sheep, and goat are the most susceptible, and it has also been observed in the pig and dog. Chickens are immune. Nicolaier produced tetanus in mice and rabbits by subcutaneous inoculation with particles of garden-earth in 1884; and Kitasato cultivated the bacillus of tetanus in pure cultures in 1889.

Microscopical Appearances.—The tetanus bacillus is a slender rod with rounded ends, 3 to 5 μ long, and 0.3 to 0.5 μ wide, and may appear as single rods or in cultures as long threads.

Motility.—Motile, although not actively so; the flagella are attached somewhat similarly to those of the bacillus of malignant oedema.

Staining Reactions.—It stains with the ordinary anilin stains, and by the Gram and Claudius methods.

Spore-Formation.—Spores are formed in thirty hours at 37° C., and at room-temperature in about a week. The spores are situated at one end of the rod, and have a diameter of 1 to 1.5 μ , giving the rod the appearance of a drumstick (see Fig. 56, \times 1300).

Excluded from air and light, the spores in a culture remain living and virulent for over a year. They can also resist heating to 80° C., but exposure to steam at 100° C. kills them in five to eight minutes. They resist the action of 5 per cent. carbolic acid for ten hours, but succumb when exposed for fifteen hours; but if 0.5 per cent. hydrochloric

acid is added, they are destroyed in two hours. Corrosive sublimate 1 to 1000 kills them in three hours, and when 0.5 per cent. hydrochloric acid is added they are killed in thirty minutes.

Biological Characters.—The tetanus bacillus is anaërobic. It grows well in an atmosphere of hydrogen, but not in one of carbon dioxide.

Kitasato, who first isolated the bacillus in pure cultures, adopted the following method :—

Cultivation.—Inoculate several white mice with wound-secretion from a typical case of tetanus. The material usually contains other organisms besides tetanus bacilli, causing more or less suppuration at the seat of inoculation in the mice.

To separate the tetanus bacillus from others present, smear the pus upon several oblique serum and agar tubes, and place at 37° to 38° C. After twenty-four hours all the organisms will have developed, and microscopic examination will reveal the presence of a few tetanus bacilli, recognisable by their shape, like a small pin, the spore representing the head. After the culture has remained forty-eight hours at 38° C., it is subjected to a temperature of 80° C. in a water-bath for forty-five to sixty minutes. A series of cultures are now prepared in media containing 1.5 to 2.0 per cent. glucose.

Kitt obtained pure cultures without using the heating process.

The original material is rubbed up with sterile water, and the liquid inoculated by means of stroke cultures on the surface of horse or sheep serum, the cultures being placed in an atmosphere deprived of its oxygen by Buchner's method (see p. 82).

On Gelatine the colonies grow slowly, the central portion being of a golden yellow colour, with numerous threads radiating from the centre.

On Agar the colonies are very characteristic, the naked-eye appearance being that of fine, fleecy clouds, which under the microscope resemble a tangled mass of fine threads. The extraordinary fineness of the latter enables the colonies to be distinguished from other anaërobic organisms.

In Gelatine Stab-Cultures the growth has the appearance of a cloudy, linear mass with outgrowths radiating into the gelatine from all sides; liquefaction follows slowly, with a coincident production of gas having an unpleasant empyreumatic smell.

In Agar Stab-Cultures the growth has a very characteristic appearance, resembling a fir-tree (see Fig. 55).

Bouillon is densely clouded.

In Milk the bacilli grow without causing any changes.

On Potatoes a moist invisible growth similar to that of the *Bacillus typhi abdominalis* occurs.

Pathogenesis.—Mice inoculated with a minute quantity of pure culture of the tetanus bacillus develop tetanic symptoms in twenty-four hours, death occurring in two to three days. Rats, guinea-pigs, and rabbits are similarly affected, but require larger doses than mice. A fatal dose for a rabbit is 0.3 to 0.5 c.c. of a well-developed bouillon culture. The period of incubation for rats and guinea-pigs is twenty-four to thirty hours, and for rabbits two to three days. Pigeons are but slightly, if at all, susceptible.

The tetanic convulsions appear first in the neighbourhood of the point of inoculation, spread thence, and finally become generalized.

At the autopsy there is usually only a small hæmorrhagic spot at the point of inoculation. No other changes are present, and the bacilli are only found at the point of inoculation (see Fig. 57), although, according to Schnitzler, they are sometimes found in the lymph-glands which are in direct relation with the inoculated part. It has been found that tetanus can be conveyed from an animal dead of it to a healthy animal by transplanting from one to the other portions of the lymph-glands near the seat of infection.

Death results from the absorption of a soluble poison which has been isolated and studied. When cultures of the tetanus bacillus are filtered through porcelain, the filtrate contains the soluble poison, which, when injected into animals, causes tetanus. Animals inoculated with pieces of the organs of animals dead from the action of the tetanus poison are unaffected; but inoculation with the blood or pleural exudates produces positive results. The poison is therefore largely present in the circulating fluids.

Vitality.—The greatest amount of poison is produced in fresh, neutral, or very slightly alkaline bouillon. The poison loses its activity when exposed for one and a half hours to 55° C., twenty minutes to 60° C., or five minutes to 65° C. When dried at the temperature of the body with access of air, the poison is destroyed; but dried at ordinary room-temperature, or at a similar temperature in the

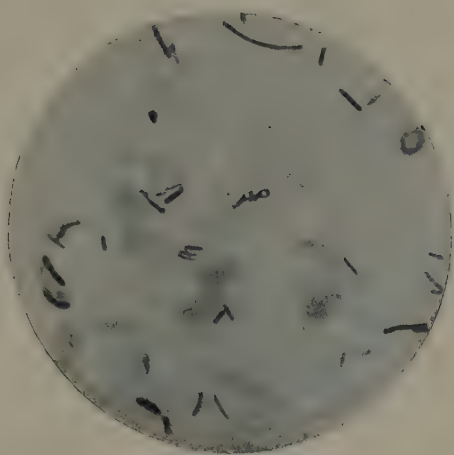


FIG. 57.—*B. tetani*. Cover-glass specimen from point of inoculation. Claudius stain. $\times 1000$.

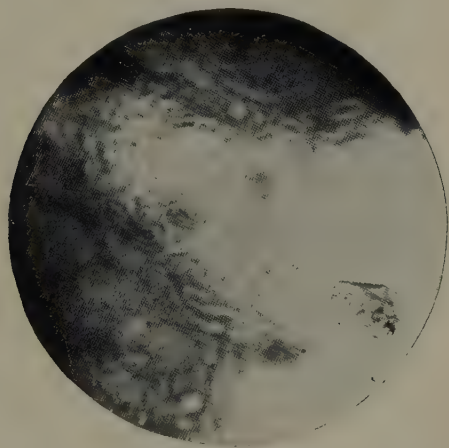


FIG. 59.—*B. mallei*. Section of glanders nodule from horse, showing single bacillus. Löffler's stain. $\times 1000$.

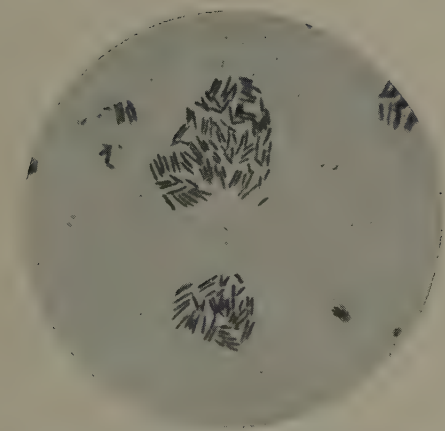


FIG. 58.—*B. proteus vulgaris*. Contact specimen from gelatine plate (agglomerations). Fuchsin. $\times 1000$.

desiccator over sulphuric acid, it is not destroyed. Diffuse daylight diminishes the virulence of the poison. Its intensity is preserved for a much longer time when kept in the dark. Direct sunlight destroys its poisonous properties in from fifteen to eighteen hours. When diluted with a fixed amount of water or bouillon, its activity is not diminished. Mineral acids and strong alkalies lessen its toxicity.

In man, as in animals, tetanus is a toxic disease. The bacilli are never found in the blood or organs, but remain localised at the point of inoculation, or in the lymph-glands about the seat of infection. The period of incubation may last from one to twenty-two days. The shorter the time between the infection and the appearance of tetanus, the more pronounced is the course of the disease, and the worse the prognosis. In cases where the incubation period of the disease was one to ten days, only about 3 per cent. recovered. When the period of incubation lasted ten to twenty-two days, 25 per cent. recovered. When the incubation period is longer, as high as 50 per cent. may recover.

Immunity and Cure of Tetanus with Anti-tetanic Serum.—

Behring produced immunity in mice by means of bouillon-cultures of tetanus bacilli weakened by adding trichloride of iodine. Anti-tetanic serum can also be obtained from horses immunised against tetanus in a similar manner to that employed in the production of diphtheria anti-toxin. The animal selected is a young horse in good condition, which is first tested with mallein, and then with tuberculin, to insure its freedom from glanders and tuberculosis. "The tetanus bacillus is grown in a bouillon culture for four or five weeks, filtered through porcelain, and a small quantity of the filtrate, about half a drop, injected into the horse subcutaneously; the dose is again repeated in three days, and if no signs of tetanus appear, in about three days 1 drop is again injected, and the process repeated for several months with continually increasing doses of the filtrate, until the horse's system becomes so resistant to the toxine that large quantities can be injected without ill effects. Six months after the first injection, the horse will probably remain unaffected by a dose of 2 ounces, and at the end of twelve or eighteen months, as much as a pint can be injected with impunity. At frequent intervals during this process, small quantities of blood are removed from the horse, and the potency of the serum tested experimentally on mice and guinea-pigs. When

it is found to confer sufficient immunity, the horse is periodically bled by piercing the jugular vein with a trocar connected by sterilized tubing with a sterilized glass flask. The blood obtained is allowed to coagulate, and its serum is separated and filtered for use. When the blood of the horse once acquires a sufficient protective power to be serviceable, the animal will continue to supply potent serum for a long and varying period without further dosing ; but the supply must be continually tested experimentally.”*

There are two other preparations in the market at present. One is a dry or powdered preparation which is dissolved in distilled water warmed to not over 40° C. As a curative agent in horses it is injected intravenously, as it is claimed that injection direct into the circulation yields results twenty-four hours earlier than subcutaneously. The second preparation is delivered in solution, and is used as a prophylactic against tetanus in man and animals, the anti-toxin being used in cases where there is a likelihood of the disease developing. The dose (0.5 to 5 c.c.) is regulated according to the time that has elapsed since the wound was inflicted. In such operations on animals as are often followed by tetanus, *e.g.*, before castration, docking, etc., 0.2 c.c. is a sufficient preventive dose.

In some parts of France where tetanus is very prevalent, Nocard distributed anti-tetanic serum for preventive purposes to sixty-three veterinary surgeons, who treated 2727 animals with it. Only one animal became affected, and this horse was not treated with anti-toxin until five days after being pricked in shoeing. Although the delay in this case was too great to prevent the appearance of tetanus, the disease ran only a mild course. During the same period these veterinary surgeons saw 259 cases of tetanus in animals that were not so inoculated.

* S. Villar, *Proc. Royal Counties Vet. Assoc.*, November 1897.

DIFFERENTIAL DIAGNOSIS TABLE.

	ANTHRAX.	MALIGNANT ŒDEMA.	TETANUS.	SYMPTOMATIC ANTHRAX.
(1) Where the bacilli are found in the animal	In the blood and organs in large numbers	Particularly near the seat of infection and in the bloody œdema	Scanty in the wound secretion; never in the blood; sometimes in the nearest lymph-glands to the seat of infection	In the bloody serous exudation at the seat of infection and in the carcase
(2) Characteristic appearance of the organism in the tissues, blood, etc.	Rods always without spores; capsules can be demonstrated in cover-glass specimens	In single rods with rounded ends, and sometimes in long jointed threads	Forms spores in the body, and occurs in rods and threads with the peculiar end spore formation or <i>drumstick</i>	Forms spores in the body, which are situated either at the end or middle of the rod, giving it a club shape; sometimes also forms short threads
(3) Motility	<i>Non-motile</i>	Motile, but <i>not always</i>	Motile, principally vegetative forms, without spores	Motile, principally vegetative forms, without spores
(4) Growth	<i>Aërobic</i>	<i>Anaërobic</i>	<i>Anaërobic</i>	<i>Anaërobic</i>
(5) Stab-Cultures	Growth, seldom without hair-like formations	Mostly without hair-like formations, also in isolated ovoid colonies	Generally like a <i>fir tree</i>	Growth in gelatine resembles a hairy caterpillar
(6) In milk	Coagulation	Coagulated slowly	Coagulation absent	Coagulated quickly
(7) Fermentation	Absent	Present	Present	Present
(8) Reaction towards the Gram stain	Stains well	Only by a prolonged exposure to the staining reagents	Stains well	By prolonged exposure to the staining reagents
(9) Pathogenic for the following experimental animals	Mice, guinea-pigs, and rabbits	Mice, guinea-pigs, and rabbits	Mice, guinea-pigs, and rabbits	<i>Not for rabbits</i>

BACTERIA ASSOCIATED WITH MEAT-POISONING.

BACILLUS BOTULINUS (VAN ERMENGEM).

This organism, which is a cause of meat-poisoning or botulism, was discovered by Van Ermengem during an epidemic at Ellezelles in Belgium. Under the term "meat-poisoning" two complex sets of symptoms, with different clinical manifestations, are included, which are differentiated by the prevailing symptoms. The one form, described as *gastro-intestinal*, appears as a cholera nostras, a simple or hæmorrhagic gastro-enteritis, accompanied with fever, albuminuria, and skin eruptions of varying form and intensity. The gastro-intestinal symptoms occur after eating tainted meat, or meat from animals slaughtered while suffering from pyæmia, septicæmia, and

puerperal fever. The organisms mostly identified as the cause of these symptoms belong to the *Bacillus coli* group, and more rarely in some cases to the *Bacillus proteus* group of organisms. The second form, known as botulism, is characterised by pronounced nervous symptoms of central origin, secretory and motor disturbances, suspension of salivary secretion, dryness and redness of the mouth and pharyngeal mucous membrane, difficulty in swallowing, hoarseness, mydriasis, ptosis, etc. The symptoms appear after the consumption of certain kinds of blood and liver-sausages, salt fish, smoked meat, hams, preserved meats, etc.

Microscopical Appearances.—The *Bacillus botulinus* occurs in the shape of large rods 4 to 6 μ long, 0.9 to 1.2 μ broad, with slightly rounded ends. The formation of threads is seldom observed, but involution forms are frequent. They are rarely found in the blood and organs of infected animals, being mostly situated at the point of inoculation.

Motility —Slightly motile, possessing four to eight flagella.

Staining Reactions.—They stain readily, and positive results are obtained with the Gram method when the alcohol is not allowed to work too long during the process of decolorizing.

Spore-Formation.—Spores are formed in cultures and in the body, they are ovoid in shape, and situated usually at the end, very seldom in the centre of the rod.

Vitality.—They are destroyed by a temperature of 85° C. in fifteen minutes, and at 80° C. in an hour. Five per cent. carbolic acid destroys them in less than twenty-four hours. Dried spores exposed to diffuse daylight live three months, and possibly longer.

Biological Characters.—The *Bacillus botulinus* is strictly anaërobic, and grows best between 20° and 30° C.; at over 35° C. spores are no longer formed, the growth is not so luxuriant, and involution forms appear. The culture medium must be decidedly alkaline, and the addition of 2 per cent. grape-sugar favours growth.

On Gelatine Plates, in four to six days round, transparent, brownish-yellow colonies develop, having a thick, lustrous, granular appearance. The colonies are surrounded by a small liquefied area; later the margins of the colony become irregular and radiating; finally they give off variously shaped processes.

In Gelatine Stab-Cultures round, whitish growths occur along the course of the needle, from which processes sometimes extend into the surrounding medium, the gelatine is liquefied, and gas is quickly formed.

Grape-Sugar Bouillon is densely clouded.

In Milk there is a slight growth, without any alteration of the medium.

On Potatoes there is no growth. The cultures give off a smell of butyric acid.

Pathogenesis.—Guinea-pigs, mice, and monkeys are susceptible. One or two drops of a liquefied gelatine culture, given on a piece of bread or in milk, is sufficient to kill the animals. Cats can be fed with large doses without being affected, but when inoculated with large doses (5 to 10 c.c.) they die in one to two days, and with small doses (1 to 2 c.c.) in eight to twelve days.

The incubation-period lasts about thirty-six hours, after which the animals become depressed, do not care to move, refuse their food, and on the third day appear stupid, the eyes almost motionless, and the pupils greatly dilated. The enlargement of the pupil increases to an enormous extent in the next few days. The tongue hangs out of the mouth, and finally the animal cannot retract it; the fæces and urine are withheld, death usually occurring from paralysis of the respiratory and circulatory organs. Very small doses of the bacilli cause marasmus, the cats dying in several weeks with symptoms of paralysis and degeneration of the internal organs. Pigeons receiving 1 to 2 c.c. of a culture first exhibit paralysis of the wings, and finally general paralysis.

The pathological changes usually present are,—a more or less well-marked hyperæmia of most of the organs; in some instances acute, sometimes interstitial, and sometimes parenchymatous hepatitis, with fatty degeneration, and desquamative parenchymatous nephritis, fatty degeneration of the heart muscle, and also of the muscles of the eye. The degenerative changes found in the central nervous system are of especial interest. In the spinal cord the changes are confined almost entirely to the grey substance of the cord along the anterior horns. In the medulla oblongata the ganglion of the hypoglossal nerve, the dorsal ganglion of the vagus, the middle small-celled ganglion of the *motores oculorum*, and of other cranial nerves are affected. Cultures obtained from fresh organs of animals

inoculated intravenously do not exhibit a very pronounced growth, but if the organs are previously placed in the incubator at 30° C. for twelve to twenty-four hours, numerous bacilli can be isolated. The *Bacillus botulinus* does not cause a genuine infection, but an *intoxication*. The toxin can be precipitated with alcohol, tannic acid, and neutral salts. Other organisms found associated with the *Bacillus botulinus* appear neither to increase nor to lessen the amount of its poisonous products.

Bacteriological Diagnosis.—In one of Van Ermengem's cases the spores of the *Bacillus botulinus* were found in a ham, mostly in the red or lean part, seldom in the fat. For animal experiments, take four parts of the suspected meat and cut it into small pieces, and add five parts of sterilized water, and inject a minute quantity of the infusion into an animal. Van Ermengem was able to isolate the bacillus from the spleen, stomach, and intestinal contents of a man dead from meat-poisoning.

Immunity.—Kempner produced immunity in animals with the filtrate of a bouillon culture. The serum of the immunized animals was highly anti-toxic, and doses of 1 to 5 c.c. injected three to twenty-four hours after a guinea-pig was poisoned with the bacillus resulted in the recovery of the affected animals. Kempner and Pollack's investigations show that after twenty hours the intoxication causes changes in the central nervous system, which again becomes normal when the serum is used.

BACILLUS ENTERITIDIS.

This bacillus was discovered by Gärtner in 1888 in a meat-poisoning epidemic, and since then has been observed in a number of similar cases.

Microscopical Appearances.—Small rods. Hanging-drop specimens from gelatine cultures show a difference between the centre and the ends of the rods, the former appearing to consist of a less refractive substance. This peculiar difference appears only to exist in gelatine cultures.

Motility.—Motile, possessing 2 to 5 long flagella.

Staining Reactions.—When stained with the ordinary anilin dyes, the middle of the rod is strongly stained, while the ends are

either weakly stained or entirely uncoloured. The reaction with the Gram method is negative.

Biological Characters.—It grows best in the incubator, but also at room-temperature.

On Gelatine Plates the colonies developing on the surface of the medium appear as thin transparent films. The gelatine is not liquefied.

In Gelatine Stab-Cultures the growth extends along the whole length of the stab, a film developing on the surface; old cultures give off a slight odour.

On the surface of Agar at 37° C. a copious grey transparent coating occurs, and a slight odour is given off.

On Potatoes at 37° C., in two days a somewhat copious greyish-white shiny coating develops.

Bouillon cultures exhibit strongly-marked uniform cloudiness.

Grape-Sugar Bouillon exhibits the same changes as occur with *B. coli*; the medium becomes strongly acid, and gas is formed—sometimes CO_2 and sometimes a combustible gas.

In Milk-Sugar Bouillon no acid reaction occurs; the fluid remains alkaline, but sometimes, although not always, small gas bubbles are found, consisting of CO_2 or a combustible gas.

Milk is not altered in chemical reaction nor coagulated.

Indol is not formed in cultures twenty-four hours at 37° C.

The bacillus grows excellently under aërobic conditions in ordinary nutrient media, but only under anaërobic conditions in the presence of grape-sugar.

Pathogenesis.—Mice and guinea-pigs are very susceptible to subcutaneous inoculation; rabbits are less susceptible to infection. Mice die in one to three days, guinea-pigs in about five days, the inoculated bacteria being found in the heart's blood and organs. Mice die in five to eight days when fed with food containing the bacillus, and the specific organism is found in the internal organs. Guinea-pigs may also be infected by way of the digestive tract. In man, the bacillus causes more or less intense gastro-enteric symptoms and occasionally death, the bacilli being found in the organs. They appear to be introduced into the intestinal canal with the food; very probably infected meat is obtained from animals in which the bacillus was present already during life.

BACILLUS MORBIFICANS BOVIS.

This organism was found by Basenau in a cow affected with puerperal fever.

Microscopical Appearances.—Rods about the same size as the typhoid bacillus, 0.3 to 0.4 μ wide, and 1 to 1.2 μ long, sometimes arranged in pairs.

Motility.—Actively motile.

Spore Formation absent.

Staining Reactions.—Easily stained with the ordinary dyes, but not by the Gram method.

Biological Characters.—The developing colonies resemble those of *B. coli*, but are more granular.

In Stab-Cultures and on *Agar* greyish-white tufts.

On Potatoes a moist yellow coating which never becomes brown.

Bouillon is clouded, a film forming on the surface.

Milk is not coagulated.

Grape-Sugar is slightly fermented, but *Cane-Sugar* remains unaltered.

Vitality.—Cultures are killed when heated one minute at 70° C.

Pathogenesis.—Mice, rats, guinea-pigs, and rabbits are very susceptible to infection by subcutaneous and intraperitoneal inoculation, also by feeding. Dogs and cats are immune. The bacillus is probably also pathogenic for man, as many cases of sickness have been observed to follow the consumption of the flesh of animals affected with puerperal fever (Ostertag).

THE PROTEUS GROUP OF BACTERIA.

This group of organisms was discovered by Hauser in 1885, and includes three species: *Proteus vulgaris*, *Proteus mirabilis*, *Proteus zenkeri*.

Proteus vulgaris.

Microscopical Appearances.—Small rods of various sizes, generally occurring in pairs, but sometimes arranged in filaments. Involution forms frequent, the most common being spherical bodies about 1.6 μ in diameter.

Motility.—Actively motile, possessing numerous flagella (see Photomicrograph, Fig. 60).

Spore-Formation absent. The bacilli are killed by five minutes' exposure to a temperature of 55° C.

Staining Reactions.—The bacilli are easily stained with fuchsin, not so easily with the ordinary watery solutions of the dyes. By the Gram method the reaction is negative.

Biological Characters.—It is a facultative anaërobe, and at ordinary room and incubator temperature the growth is equally luxuriant, the optimum temperature being 20° to 25° C.

On Gelatine Plates small, round, yellowish colonies with thick centres and irregular edges develop at first, from which brush-like offshoots are thrown out. Other colonies are surrounded by a zone of threads which, partly in circular, partly in irregular twisted forms, surround the central opaque mass. The gelatine is quickly liquefied. Straight and twisted offshoots, which frequently become detached from the mother-stem, grow into the surrounding medium, and continue moving in the somewhat softened gelatine. This condition is known as "*Swarming Islands*" (see Photomicrograph of same, Fig. 58), and is easily observed in cultures on 5 to 6 per cent. gelatine. The growth may give rise to peculiar figures and designs, for which reason the bacillus has also been designated the *Bacillus figurans*.

Stab-Cultures in gelatine liquefy very quickly.

On Agar slants a rapidly extending moist grey layer is formed.

On Potatoes a dirty greyish coating develops.

Bouillon becomes uniformly clouded. Cultures on all the different media give off an abominable smell.

Pathogenesis.—When a considerable quantity of a proteus culture (3 c.c.) is injected intravenously, or into the peritoneum of a rabbit or guinea-pig, the animal dies of acute enteritis and peritonitis. The intravenous injection of 5 to 10 c.c. of a bouillon culture in dogs causes more typical symptoms and lesions. The symptoms produced are bloody vomiting and diarrhœa, combined with severe tenesmus and elevation of temperature. At autopsy an intense hæmorrhagic enteritis is found. The blood and internal organs either contain none or very few bacilli. With filtered cultures, and cultures containing the remains of dead bacilli, the same results are obtained. The proteus is also

fatal to mice, and the bacilli can again be cultivated from their organs. The oftener the bacillus is passed through mice the more virulent it becomes.

The proteus is found in all putrefactive processes, and in the intestinal canal. In man it may give rise to mixed infection with the ordinary exciters of inflammation. It causes the ichorous, putrid phlegmon sometimes observed in cases of cadaveric infection. The proteus, further, sometimes penetrates suppurating wounds, and multiplying there, forms toxic products which, when absorbed, cause the so-called "putrid intoxication." According to Jäger, certain forms of icterus, accompanied with fever, pain in the muscles, and enlarged liver and spleen, known as "Weil's disease," are produced by the proteus. Jäger was able to cultivate a fluorescent proteus from the urine, and, after death, from the organs of individuals dead of Weil's disease. The infection resulted in these cases from bathing in river-water which was contaminated with proteus. An outbreak of disease due to the *Proteus fluorescens* also occurred among some poultry kept on the banks of a small stream.

Levy found the proteus to be the cause of a hæmorrhagic gastro-enteritis which appeared in seventeen persons after partaking of decomposed meat. According to Booker, the *Proteus vulgaris* plays an important part in the production of the symptoms characteristic of cholera infantum. It was found in the alvine discharges of affected infants, but not in those of healthy infants. The prominent symptoms in the cases where the proteus was found were drowsiness, stupor, emaciation, more or less collapse, frequent vomiting and purging, with watery and generally offensive stools.

Bacteriological Diagnosis.—Plate-cultures are prepared from the pus, from the ichorous phlegmons, and also from the urine obtained under aseptic precautions from the patients affected with Weil's disease, etc.

Proteus mirabilis.

Microscopical Appearance.—Rods of various lengths, the smallest being about $0.6\ \mu$ long.

Spore-Formation absent.

Biological Characters.—On *Gelatine Plates* the deep-lying colonies exhibit curiously formed, twisted zoöglæa masses. The



1.

2.

FIG. 61a.—GLANDERS. Nasal Lesions in Horse.

1. Typical ulcers on nasal mucous membrane.
2. Extensive ulceration and necrosis of mucous membrane.

T. Bowhill, F.R.C.V.S., Photo.]

[*Face page 162.*

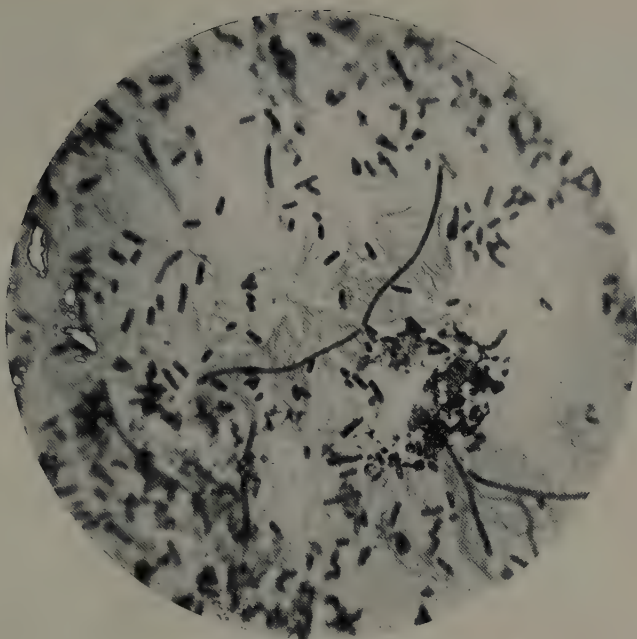


FIG. 60.—*B. proteus vulgaris*, showing flagella.

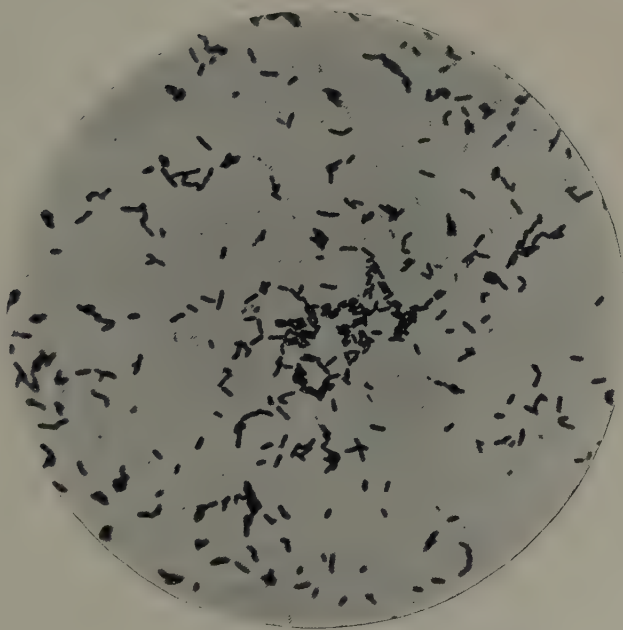


FIG. 61.—*B. mallei*. Glycerine-agar culture. Stained with fuchsin. $\times 1000$.

surface colonies occasionally form "*swarming islands*" like the *Proteus vulgaris*. Gelatine is liquefied slowly.

Proteus zenkeri.

Microscopical Appearance.—Bacilli $0.4\ \mu$ broad, and averaging $1.6\ \mu$ long.

Spore-Formation absent.

Biological Characters.—It occasionally forms "*swarming islands*," like *Proteus mirabilis*; but the gelatine is *not liquefied*.

BACILLUS MALLEI.

(The Glanders Bacillus; *Ger.* Rotzbacillus; *Fr.* Bac. de la Morve.)

This organism was discovered by Löffler and Schütz in 1882 in the diseased tissues of animals affected with glanders. It was isolated in pure cultures, which, when inoculated into susceptible animals, reproduced the disease with all its clinical and pathological manifestations.

Microscopical Appearances.—Short rods, 2 to $3\ \mu$ long, 0.2 to $0.4\ \mu$ thick, with rounded or slightly pointed ends, generally occurring in single rods, sometimes in pairs, and very seldom in long filaments.

Motility.—Non-motile.

Staining Reactions.—The bacilli stain readily in cover-glass specimens with the ordinary basic anilin dyes (see Photomicrographs, Figs. 59 and 61). The staining is somewhat irregular, the bacilli presenting a granular aspect, owing to alternating clear and uncoloured spaces (for special Staining methods, see p. 47). The bacillus is not stained by the Gram method.

The bacilli are always present in diseased tissues, although great difficulty is usually experienced in demonstrating them by staining methods. When properly stained, they are found most numerous in the centre of the nodules, becoming gradually less so towards the periphery. Their usual position is between the cells, but sometimes they almost fill some of the epithelial cells. They are always present in the nodules (see Photomicrograph, Fig. 59), rarely in the blood, and if so, only in small numbers.

Spore-Formation.—Unknown.

Biological Characters.—The bacillus is a facultative organism, growing both in the presence and absence of oxygen. It develops

on the ordinary nutrient media. Minimum temperature, 25° C.; optimum, 37° C.; maximum, 42° C. The best growth is exhibited on 5 per cent. glycerine-agar.

On Agar, with or without the addition of 5 per cent. glycerine, it forms a moist, opaque, glazed layer, devoid of special characteristics. Sometimes no growth occurs on ordinary agar.

On Blood-Serum it forms a moist, opaque layer of yellowish or dirty-brownish drops. The serum is not liquefied.

On Potatoes the bacillus exhibits a most characteristic growth, which is somewhat rapid, and in twenty-four to thirty-six hours at 37° C. a moist, amber-yellow, transparent growth appears, becoming deeper in colour and denser in consistency, until it finally presents a reddish-brown colour, and the surrounding surface of the potato becomes darkened. According to Semner, the bacillus exhibits unusual pleomorphism on potatoes, often forming long felt-like interlaced filaments, not unlike the threads of *Bacillus anthracis*, and finally, blebby and club-like swellings.

According to Kresling, if the potatoes have been frost-bitten, or have begun to bud, growth is entirely checked by the production of much acid owing to the presence of sugar in such potatoes. The bacillus produces acid in solutions containing grape or milk-sugar.

In Bouillon a diffuse clouding takes place, a tenacious, ropy sediment being ultimately formed.

In Litmus-Milk the blue colour becomes reddish in four or five days, and quite red in two weeks, at 37° C., the milk being finally separated into a firm clot of casein and clear whey.

Vitality.—Cultures of the *Bacillus mallei* lose their virulence after the fourth and fifth generations; therefore to retain the cultures virulent it is necessary after two or three culture generations to pass the virus through a susceptible animal. According to Löffler the bacillus lives three months in a dry condition, while other authors find that when spread out in a thin layer the bacilli die in ten days. When exposed to heat they are killed at 100° C. in two minutes, and at 80° C. in five minutes. Exposed to the action of corrosive sublimate $\frac{1}{1000}$ they are killed in fifteen minutes, and in 5 per cent. carbolic acid in one hour; and they also lose their virulence quickly in distilled water (six days). Their virulence is not destroyed by putrefaction; inoculations made with central portions of glanders lungs exposed to the air for fifteen to twenty-six days have given positive results.

Pathogenesis.—The ass, mule, horse, goat, cat, sheep, dog and pig, and man are susceptible. Cattle are immune. Among laboratory animals the field mouse, wood-mouse, and the guinea-pig are the most susceptible, the rabbit being much less so; white mice and house mice are immune. Birds, with the exception of the pigeon, are refractory.

Field mice inoculated subcutaneously with a small quantity of a culture die in three to four days. The spleen is found enlarged, and is generally studded with minute grey nodules, rarely present in the lungs, but frequently found in the liver. Pure cultures can be obtained from these nodules. The characteristic lesions are much more marked in the guinea-pig, which lives from six to eight weeks after inoculation. The specific inflammatory condition of the mucous membrane of the nostrils is almost always present; the joints are infiltrated and swollen. Orchitis and epididymitis are present in male animals, while the internal organs, lungs, kidneys, spleen and liver are generally the seat of the characteristic nodular formations. Pure cultures can be obtained from the diseased tissues.

Modes of Infection and Course of the Disease.—Nocard found that miliary tubercles identical with those found in cases of natural glanders, developed in the lungs of horses fed on cultures, pus, etc.

Prieur, according to Nocard, gives the most complete and most lucid dissertation of the actual state of our knowledge of the farcoglanderous affection (*Veterinary Record*, 1898, p. 505).

Nocard believes that the glanders virus commonly gains entrance into horses by means of the alimentary canal. Translucid tubercles are of a glanderous nature. Certain forms of cutaneous glanders may be cured in man by means of energetic and prompt treatment.

Certain cases of pulmonary glanders in the horse may end in recovery by virtue of the natural resistance of the animal which is increased by good hygienic surroundings.

The employment of mallein is the sole means of diagnosis which we have at our disposal in cases of glanders exempt from clinical signs. An animal which has given a *complete* reaction to mallein and does not react again after a variable number of injections of the reagent, may be considered as cured. Rigid application of the rules of sanitary police remains the most efficacious measure of preventing glanders in man and animals.

In man a local swelling appears at the seat of infection. The

swelling spreads quickly, and is accompanied by suppuration and cording of the neighbouring lymphatics. Multiple abscesses are next formed in the skin, muscles, and internal organs, and there are often suppurative changes in the joints; at this stage the disease resembles pyæmia. Characteristic glanders nodules appear in the mucous membranes, particularly in the nose, and these soon disintegrate, giving rise to ulcers. Death is caused by general infection, carried by means of the lymph circulation.

Löffler observed a female guinea-pig which resisted a glanders inoculation, and five months after being inoculated gave birth to one young one, which at birth seemed perfectly healthy, but died in a week from glanders of the viscera.

Bacteriological Diagnosis of Glanders.

Straus's Test.—Inoculate a male guinea-pig in the peritoneal cavity with a suspension of suspected material or culture, making the inoculation directly in the middle line of the abdomen, otherwise other bacteria may be introduced into the vesiculæ seminalis, and cause orchitis, etc. If the material inoculated is from a genuine case of glanders, the testicles commence to swell in thirty hours, and the skin over them becomes hyperæmic, shiny, and finally desquamates, evidence of the formation of pus appears, and the purulent matter often breaks through the skin. The diagnostic symptom is the tumefaction of the testicles.*

Mallein.—This consists of the filtered products of the glanders bacillus, and is analogous to tuberculin. It may be prepared from old glycerine-bouillon cultures of the glanders bacillus by steaming them for several hours in the sterilizer, or in the autoclave for fifteen minutes at 115° C., and filtering through unglazed porcelain. The filtrate is concentrated to one-sixth its volume, and mixed with an equal volume of $\frac{1}{2}$ per cent. solution of carbolic acid. This yields an active mallein, the dose being 1 c.c., and it gives good reactions.

According to Hunting (*Veterinary Record*, 4th December 1897):—“With mallein diagnosis is easy, and in ninety-nine out of a hundred cases is certain. An injection of mallein under the skin of a healthy horse has no effect, or at most it produces a swelling as big as a watch at the point of injection. An injection into a glandered horse produces two reactions—a large and painful swelling at the point of injection, and a rise of temperature to 104° or even 106° Fahr. The indications of mallein are not always so exact that it can be used without

* See further under *B. orchiticus*, which also produces an orchitis in guinea-pigs.

brains. It will not do to say no horse is glandered until the temperature rises 4° and a swelling appears within twenty-four hours measuring 5 inches across. Sometimes the local swelling is less, and sometimes the temperature does not rise much. When the temperature is already 103° F. a rise may not take place at all; but in such a case a painful swelling at the point of injection is conclusive evidence. In hundreds of cases I have proved the trustworthiness of mallein, when no outward sign of disease existed, by post-mortem results. In hundreds of healthy horses I have known it used without one ill effect." These conclusions are of immense practical and diagnostic value, owing to Hunting's extensive practical experience with glanders and the uses of mallein in London.

Nocard recommends that only animals presenting *clinical* signs and reacting to the mallein test should be slaughtered. The other animals should be isolated and submitted every month or two months to the mallein test, and when they have supported two tests without reacting, they can be placed at the free disposal of the owners, for they will then have completely and definitely recovered from the glanders lesions which they carried in their lungs. Nocard further states that recovery is far from rare. The glanders nodules found on post-mortem examination of such cases did not produce disease when inoculated into susceptible animals, nor could any diagnostic cultures be obtained.

Hamilton* has recently obtained results somewhat analogous to Nocard during the testing of the Glasgow Corporation's stud of tramway horses, with mallein.

The author had occasion recently at Cape Town, South Africa, to test a number of mules with mallein (using double the ordinary dose), and observed that injections of mallein, repeated at intervals, undoubtedly exhibited a curative effect in the early stages of the disease; while in cases of longer standing the mallein injections appeared to hasten dissemination of the specific virus throughout the body, as in some instances cutaneous and nasal lesions appeared in animals that, previous to the mallein injection, presented no clinical symptoms. These conclusions are based on the results of a number of post-mortem examinations made by Vet.-Major Cooper, and the writer, on the reacting animals.

Three mules with a slight nasal catarrh (no ulceration or glandular

* *Journal Comp. Path. and Therap.*, vol. xiii., part ii., p. 176.

enlargements being visible), that did not yield to the ordinary remedies, reacted both locally and thermally on two occasions, the nasal catarrh entirely disappearing after the second inoculation. By order of Vet.-Col. Smith these mules were again tested with mallein, and not reacting, they were branded "M" on the near shoulder, and placed at work under daily supervision. Three months later they were still healthy, and no signs of any disease were apparent in them, or in any of the other mules they were in contact with.

That the mallein test appears to be less reliable in the hybrid than in the horse was also our experience, hence the employment of double the dose required for a horse.

BACILLUS ORCHITICUS.

Found by Kutscher in the nasal discharge of a horse affected with glanders.

Microscopical Appearances.—Similar to the glanders bacillus.

Motility.—Non-motile.

Staining Reactions.—Stains with ordinary stains and by the Gram method.

Vitality.—Killed by exposure to 55° C. for five minutes.

Biological Characters.—It grows on all the ordinary media except milk.

On Gelatine Plates it forms colonies that resemble old colonies of the cholera spirillum. Liquefaction occurs somewhat rapidly at 22° C.

On Agar, the growth presents the appearance of thick white tufts.

On Blood-Serum, an orange-yellow pigment is often formed; the medium is liquefied.

In Bouillon and Peptone Solution small flakes are formed, the medium very seldom becoming clouded.

Pathogenesis.—Guinea-pigs (male) when inoculated intraperitoneally with a small quantity of the virus, exhibit swelling of the testicles in forty-eight hours, and generally die in four to five days. The principal lesions are nodules in the mesentery and testicles (rarely in the abdominal organs). Large doses cause death in one to three days with more pronounced changes in the peritoneal cavity. Small doses introduced subcutaneously cause death in one to two days, with an extensive œdema affecting the whole abdominal wall. Guinea-pigs

which have recovered are immune to renewed infection. *Mice* inoculated subcutaneously with small doses die in four to seven days, an abscess developing, the surrounding tissue being œdematous, and infiltrated with small hæmorrhages. The bacilli were only present in the pus from the abscess, and frequently within the leucocytes. *Intraperitoneal* inoculation produces death in the same time, numerous nodules being formed on the peritoneum—the liver and spleen being seldom affected. Intrapulmonary injection causes the formation of a watery hæmorrhagic effusion into the serous cavities of the thorax, and the development of numerous grey nodules on the pleuræ, associated with small lobular pneumonic centres. *Rabbits* are not so susceptible to infection, while chickens and pigeons are immune.

Differential Diagnosis.—With the Gram method of staining the bacillus of glanders gives *negative results*, while the *Bacillus orchiticus* yields *positive results*.

EPIZOÖTIC LYMPHANGITIS.

(*African, Neapolitan or Benign Farcy.*)

This is a virulent contagious disease due to the presence in the tissues of a specific *Cryptococcus* discovered by Rivolta* in 1883. This discovery was confirmed by Nocard† in 1891.

The characteristic clinical phenomena are suppuration of the superficial lymphatics. It develops from wounds, the period of incubation averaging about three months, and according to some investigators much longer. Ulcers resembling those of acute glanders were observed on the nasal mucosa of animals affected with this disease, but as Nocard demonstrated the presence of the *cryptococcus* in the lesions, the relation to lymphangitis and not to glanders was affirmed.

Microscopical Appearances.—Specimens prepared from fresh pus, and examined unstained with a power of 500 diameters, exhibit roundish bodies with pointed ends, or one end pointed and the other rounded, highly refractile, and with a double contour lying both free and enclosed in the pus-cells. The organism develops by budding.

Staining Reactions.—It is stained with difficulty by the Gram, Weigert, and Kühne methods, and after long contact with Ziehl's stain.

* Rivolta e Micellone, *Del farcino cryptococcico*, Gior. Fis. et Patol., 1883, p. 143.

† Nocard: "Sur le diagnostic de la lymphangite epizootique," *Bulletin de la Soc. de Méd. Vét.*, 1891, p. 367.

Vitality.—In pus the organism is destroyed in a few minutes when heated to 80° C. It resists the action of 5 per cent. carbolic acid (Rivolta and Micellone).

Biological Characters.—It is cultivated with difficulty. Tokishige* obtained cultures in peptone-bouillon, on gelatine, agar, and on potatoes. Gelatine is not liquefied. On agar the growth consists of slightly raised, greyish-white grains. On potatoes the growth is somewhat rapid, and of a faint brown colour. Marcone obtained cultures on horse serum, to which he added glucose, glycerine, and cane-sugar in the proportion of 2 per cent.

Pathogenesis.—Horses, mules, and cattle are susceptible. The dog, cat, calf, and the pig are entirely immune (Tokishige).

Diagnosis.—The presence of the cryptococcus in the pus from the lesions, the absence of either local or thermal reaction to the mallein test, and non-reaction to Straus's test, distinguishes this malady from glanders.

ULCERATIVE-LYMPHANGITIS.

This form of lymphangitis is caused by a specific bacillus discovered by Nocard,† and is characterised by the presence of abscesses, and ulcerated sores in the skin and along the course of the superficial lymphatics. It is of especial interest as the clinical symptoms and specific reaction of the virus in inoculated male guinea-pigs (Straus's reaction) are analogous to those of cutaneous glanders.

Microscopical Appearances.—In pus obtained from the lesions the bacilli occur as small, thick, round rods with rounded extremities, free in the fluid of the specimen or enclosed within the cells.

Staining Reactions.—It stains by the Gram method.

Biological Characters.—It is an aërobe, and grows on all the ordinary media. In bouillon and 2 per cent. solution of peptone the medium is not clouded, but on the third day a deposit is formed of small, whitish clusters of bacilli varying in size according to the age of the culture.

On Agar white colonies with projecting centres and crenated

* Tokishige, "Über pathogene Blastomyceten," *Centralbl. für Bakteriöl.*, vol. xix., 1896, p. 105 (with plates).

† Nocard, *Bulletin de la Société centr. de Méd. Vét.*, 1893, p. 116. *Ibid.*, 1894, p. 92. *Ibid.*, 1897, p. 420. Ref., Nocard and Leclainche, *Les Maladies Microbiennes des Animaux*, 2nd Edition, 1898.

margins develop, appearing later as a finely puckered, thin, moist, opaque membrane, non-adherent to the medium.

On Horse-serum the growth is white, while on ox-serum it is more or less yellow-coloured.

Milk is not coagulated.

On Potato a dry, downy, dirty white-coloured layer with a scalloped border develops; the addition of glycerine assists the development, the growth forming a moist, colourless layer.

Pathogenesis.—This bacillus is pathogenic for the horse, guinea-pig, rabbit, mouse, and pigeon.

Diagnosis.—Animals suffering from this disease do not react to the mallein test, and although the bacillus causes orchitis in guinea-pigs (Straus's reaction), the nature of the same is determined if bacilli that stain by the Gram method are present in the lesions.

The clinical phenomena in the horse differ from those found in cutaneous glanders by the total absence of induration of the lymph-glands in the affected regions, as well as the tendency to cicatrization when simple antiseptic dressings are applied. After the primary lesions heal, others appear indefinitely in successive crops in the neighbouring tissues.

DIFFERENTIAL DIAGNOSIS TABLE.

	GLANDERS.	EPIZOOTIC LYMPHANGITIS.	ULCERATIVE LYMPHANGITIS.
(1) Characteristic appearance of the organism	Short rods 2 to 3 μ long, 0.2 to 0.4 μ thick, with rounded or slightly pointed ends	Roundish bodies with slightly pointed ends occurring both free and within the cells	Small thick bacilli with rounded ends both free and lying in the pus cells
(2) Reaction with the Gram method	Negative	Stains with difficulty	Positive
(3) Effect of Straus's reaction	Positive	Negative	Positive
(4) Animals affected	Ass, mule, horse, goat, cat, sheep, dog, and pig	Horses, mules, and cattle	Horse, guinea-pig, rabbit, mouse, and pigeon
(5) Mallein reaction	Positive	Negative	Negative

BACILLUS TUBERCULOSIS.

The infectious nature of this disease was first demonstrated by Villemin in 1865, when he communicated the disease to healthy experimental animals by means of tuberculous material. Cohnheim

confirmed these experiments by inoculations into the anterior chamber of the eye in rabbits. In 1882 Koch discovered the *Bacillus tuberculosis* (see Figs. 62-69).

Microscopical Appearances.—Koch's bacilli are small, thin rods, varying in size from 0.2 to 0.4 μ broad, to 3 to 4 μ long; they are slightly bent, generally occur singly, but in cultures sometimes form chains of four to six individuals; at times they exhibit a club-like swelling at one end and branches. They have for this reason been classified by some authors as streptothrices.

Motility.—Non-motile.

Spore-Formation.—The clear spaces present in stained specimens have been described by some as spores, by others as degenerative (see Fig. 62). There is no proof as to the existence of spores.

Staining Reactions.—The bacilli stain with difficulty, but once they are stained they retain the dye with great tenacity. The results with the Gram and Claudius staining methods are positive. (For the special methods of preparing and staining cover-glass specimens and sections, see pp. 22, 23, and 46.)

According to Koch, tubercle bacilli contain two solid fatty acids, one of which is soluble in dilute alcohol, and saponified by caustic soda; the other is not saponified, and is only soluble in boiling absolute alcohol and ether. Both of these fatty acids are stained by the tubercle bacillus stains; but by the process of decolorization, the one soluble in alcohol gives up the stain, while the other retains it; thus the acid fixes the staining material and accounts for the staining reaction (see Photomicrograph of tubercle bacilli in sputum, Fig. 62). It is possible by means of a warm solution of caustic soda to remove the fatty acids from the bacilli, and to observe under the microscope how they pass out in the form of colourless drops, and coalesce into larger drops. According to Koch, these fatty acids form a continuous layer on the bodies of the bacilli, thus providing them with a protection against external influences.

This peculiar micro-chemical staining reaction found in the case of *Bacillus tuberculosis* is not confined to that organism alone, as other species of bacilli, when similarly treated, react in a somewhat similar way.

1. *The smegma bacillus*, which is found in smegma, often seen beneath the prepuce and upon the vulva, both normally and in disease.

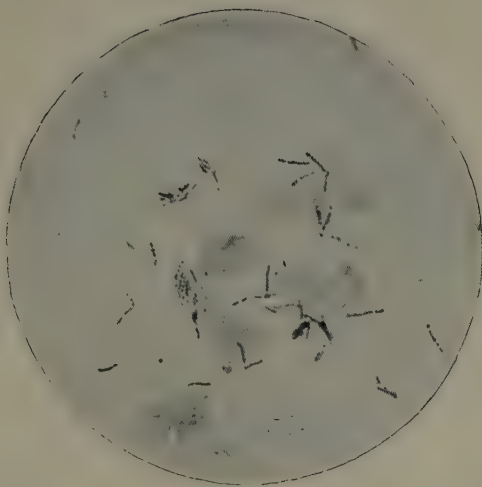


FIG. 62.—*B. tuberculosis*. Cover-glass specimen prepared from sputum. Ehrlich's stain. $\times 1000$.



FIG. 63.—*B. tuberculosis*. Glycerine-agar culture.

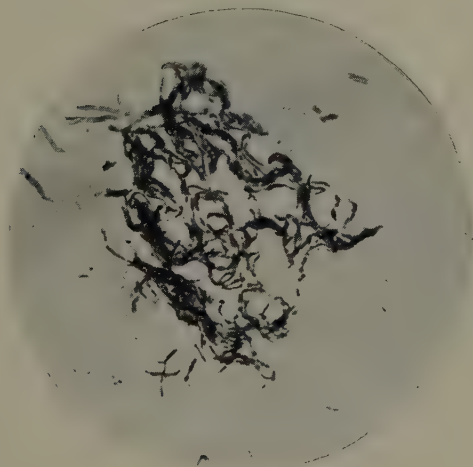


FIG. 64.—*B. tuberculosis*. Cover-glass specimen from pure culture on glycerine-agar. Ehrlich's stain. $\times 1000$.

2. *Lustgarten's bacillus of syphilis*, found principally in the primary sores.

3. *Bacillus lepræ*.

4. *The acid-resisting bacteria* found in butter.

Hueppe differentiates the first three organisms from *B. tuberculosis* as follows :—

1. Treat the preparation, stained with carbol fuchsin, with sulphuric acid, and the syphilis bacillus is decolorized almost instantaneously.

2. If not at once decolorized, treat with alcohol, and if it is the smegma bacillus it will lose its colour.

3. If it is still not decolorized, it is either the leprosy or tubercle bacillus. According to Baumgarten, the bacillus of leprosy is stained by an exposure of six or seven minutes to a cold, saturated, watery solution of fuchsin, and retains the stain when subsequently treated with acid alcohol (nitric acid one part, alcohol ten). When treated for the same length of time, the bacillus of tuberculosis does not ordinarily become stained.

Biological Characters.—It is difficult to obtain a pure culture of tubercle bacilli, because they grow slowly, and require for their development an incubator temperature of—minimum, 29°; optimum, 37° to 38°; and maximum, 41° C.

The Koch bacillus grows well on blood-serum, 4 to 6 per cent. glycerine-agar, and in glycerine-bouillon. The isolation of the tubercle bacillus from the mixture of bacteria in tubercular sputum by means of glycerine-agar plates is almost impossible*; on account of the tubercle bacilli growing so slowly, the other bacteria outgrow and overwhelm them easily. It is only possible to obtain a pure culture when the material is quite pure at the commencement. The following is the method of procedure :—

Inoculate say two guinea-pigs (very susceptible) subcutaneously or intraperitoneally with material containing tubercle bacilli. In three to four weeks kill one of these, disinfect the skin with sublimate solution ($\frac{1}{1000}$), and remove the skin from the abdomen. The peritoneum is opened with sterilized instruments, and the spleen removed with forceps previously heated in the flame. By this mode of infection the spleen is usually the most affected. A portion of tissue containing tubercles is removed from the spleen with sterilized forceps, and crushed between two sterile scalpels or glass slides. The crushed

* See p. 175.

material is now conveyed by means of a stout, sterile platinum needle on to the surface of solidified blood-serum. The whole process must be accomplished quickly and with the greatest care, because if another germ enters the serum-tube, it will soon outgrow the tubercle bacillus. As a greater precaution, several cultivations ought to be prepared at the same time. As the cultures must remain some weeks in the incubator at 37° to 38° C., they must be closed with indiarubber caps previously sterilized in sublimate solution, or the superfluous overhanging cotton-plug burnt off in the flame, and the tube closed with melted paraffin or sealing-wax. Without these precautions the water of condensation will be all absorbed and the culture media dried up.

When the inoculation is successful, signs of growth are usually observed after two weeks on the blood-serum. Small, grey, dry scales develop, which, when examined under a low power, appear to be composed of delicate twisted lines. The development goes on slowly, and in four to six weeks other tubes can be inoculated from the original culture. The growth of the second generation requires two weeks before it is distinct. Later generations, usually the fifth or sixth, grow more luxuriantly and rapidly, especially when the atmosphere of the incubator is moist, so that the use of indiarubber caps, etc., can be dispensed with, whereby the cultures are ventilated. Under such conditions, in seven to fourteen days the whole of the surface of the serum is covered with the characteristic dry scales. From the fifth serum-generation it is easy to obtain cultures on glycerine-agar. On this medium the development is abundant, the bacilli forming a greyish, dry coating of brittle, curly, slightly elevated fragments (see Photograph, Fig. 63). This coating growing downwards, covers any water of condensation present without clouding it, and when the culture is left long enough in the incubator, it grows a considerable distance up the free sides of the test-tubes, where no nutrient medium is present. Veal bouillon, with 6 per cent. glycerine added (in Erlenmeyer flasks), is the best fluid medium. When inoculating this bouillon, it is necessary to place the dry scales *upon* the fluid, so that they swim on the surface. The tubercle bacilli develop only in the upper layers or on the surface of the medium where there is oxygen. On the surface of veal bouillon, containing 6 per cent. glycerine, the tubercle bacilli form a membranous surface growth, which exhibits the same characteristics as the coating on glycerine-agar. Under favourable circumstances, in a few weeks,

sometimes in ten days, the growth extends a considerable distance up the walls of the tube ; the lower portion of the bouillon *remains perfectly clear*, which is characteristic of the growth of the tubercle bacillus.

On Potatoes, the under ends of which are immersed in a solution containing 5 per cent. of glycerine, the tubercle bacilli develop very well, forming on the surface of the potatoes thick, warty tufts, the glycerine solution remaining clear. The potatoes are prepared according to Roux and Globig's method (see Technique, p. 61).

In 1882 Kitasato devised a means of obtaining cultures of tubercle bacilli direct from the sputum of phthisical patients. The patient's mouth is washed out with an antiseptic gargle, and the sputum collected in a sterile Petri-dish. One of the expectorated masses of sputum is washed in several changes of sterile water to free the exterior of bacteria. Out of the middle of the washed sputum a portion is removed with the platinum needle, and stroke cultures prepared on the surface of blood-serum. The developing cultures exhibit a somewhat different appearance to those cultivated from the bodies of animals ; they appear as round, whitish, transparent colonies, but later generations grow in the same manner as those previously described. The bacilli can be cultivated through many generations and for several years without injuring their power of growth, although the older cultures become less virulent.

Vitality.—The *Bacillus tuberculosis* is destroyed by heating for ten minutes at 70° C., one minute at 95° C., one hour at 60° C., and four hours at 55° C. It resists the action of direct sunlight for some minutes or hours, according to the thickness of the layer exposed. Exposed to diffuse daylight it is killed in a week.

Pathogenesis.—None of the domesticated animals are immune. The guinea-pig is the most susceptible of experimental animals, a very small quantity of tubercle bacilli being sufficient for infection ; next come the rabbit and the field-mouse ; less susceptible, but still far from immune, are white mice and dogs. Young animals exhibit a greater predisposition for tuberculosis than older animals. Typical tubercular lesions are produced in guinea-pigs and rabbits by subcutaneous injection, inoculation into the anterior chamber of the eye, intraperitoneal and intravenous injection, or by inhalation of moist powdered tubercle bacilli. Susceptible animals infected by feeding with tubercular matter die of abdominal tuberculosis. Baum-

garten inoculated animals in the anterior chamber of the eye, and in three days found the bacilli in the auricular lymph-glands. Intravenous injection produces a generalized miliary tuberculosis. The experiments of Schüller are of especial interest in relation to many localized tubercular diseases of man; he injected tubercular material in an animal, then produced injury in the region of the knee-joint, and observed that the infection became localized at that point. Dead tubercle bacilli produce suppuration; they are *positively chemotactic*, attracting the leucocytes towards them. When dead bacilli, as shown by Prudden, are injected intravenously into rabbits, and the animals killed after an interval, small tubercles are found throughout the lungs and liver. These tubercles are formed of round cells, epithelioid cells, giant cells, and dead tubercle bacilli, which cannot be distinguished from the living bacillus. Baumgarten considers that the dead tubercle bacilli produce a tuberculosis similar to that produced by a foreign body (pseudo-tuberculosis).

Instances in which physicians and veterinary surgeons have contracted the disease, in making autopsies on diseased men or animals, are incontestable, although fortunately rare. The virus in such cases gains entrance by some cutaneous wound, causing at first a more or less limited cutaneous tuberculosis, which later may become generalized.

Bovine Tuberculosis.

According to most authorities, tuberculosis of the lungs (see Fig. 65) is the most frequent of all the primary forms of this disease in cattle, infection being caused by dried tubercular matter inhaled into the lungs. According to Eber, the bacilli, which certainly are often present in the fæces of tuberculous cows, may very well be a source of infection of milk which is of itself quite normal. According to Smith, the ox is much more easily and more severely infected with cultures of bovine origin than with those obtained from lesions of the human subject. Primary tuberculosis also occurs in the lymph-glands of the head and neck, in the mesenteric glands, the intestines, the liver, the genital organs, and the udder. Bang is inclined to believe that the udder is now and then primarily affected in animals that are in very good condition. Eber reports cases of primary tuberculosis of the penis, vagina, and vulva. Finally, *generalized* infection, due to the dissemination of tubercle bacilli through the blood, occurs in two forms: (1) The *acute* form,

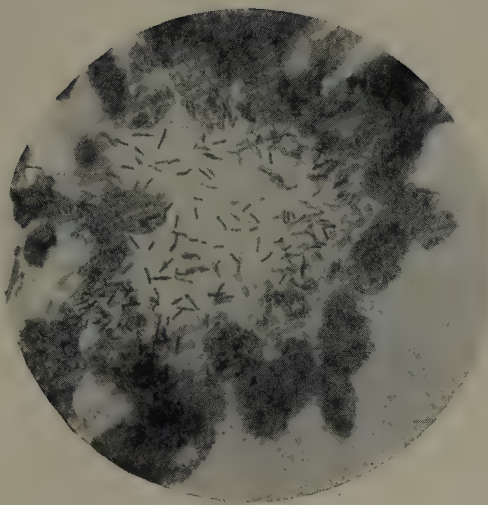


FIG. 65.—*B. tuberculosis*. Cover-glass specimen, lung of a cow.
Ehrlich's stain. $\times 1000$.

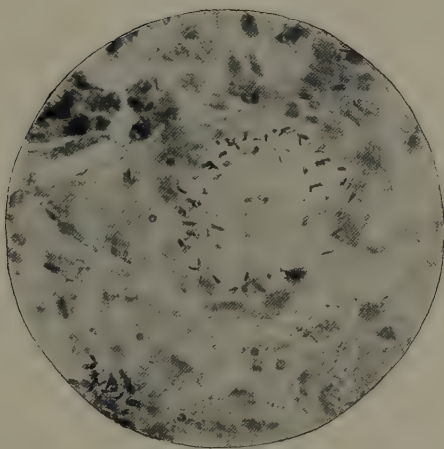


FIG. 66.—*B. tuberculosis*. Section of horse's mesenteric
gland. Ehrlich's stain. $\times 600$.

when large numbers of bacilli have escaped into the circulation, whereby numerous tubercular foci appear in various organs ; (2) The *chronic* form, as seen in old cows affected with tuberculosis for many years. A few cases of congenital tuberculosis have been recorded in calves. Johne found tubercle bacilli twice in the organs of embryos. The writer observed a case of tubercular meningitis in a calf two to three weeks old in California. The mother of this calf was tested with tuberculin and reacted, the post-mortem revealing tubercular lesions, one ovary being affected. King mentions a case of a cow giving birth to twin calves. The viscera of both, when submitted to examination, proved to be tuberculous. The cow was not tested with tuberculin. (*Proc. of National Vet. Assoc., Leeds, 1898.*)

According to Nocard, the tubercle bacillus is only in very exceptional cases transmitted from mother to foetus ; the predisposition, however, to infection is hereditary. Infection rarely takes place while diseased and healthy cattle are pastured together in the open. Nocard and other observers frequently point to the fact of the animals being infected while standing in stalls adjacent to the coughing "piner," the disease often extending along one side of the byre, while the cows on the other side may remain sound, and it is also observed that the animals longest stabled furnish the largest proportion of cases. While the writer was inspector for the city and county of San Francisco, the tubercular seizures, with a few exceptions, were all of dairy cows, tubercular lesions in range cattle being hardly ever observed.

Nocard is against the total seizure of the carcasses of cattle which have localized tuberculosis, but are otherwise in good condition. He insists that the tubercle bacillus is not present in the blood or muscles, excepting for very brief periods, and in cases of advanced general tuberculosis. In some of Nocard's experiments with meat from cases of generalized tuberculosis, he found that although it had no bad effects when eaten, the expressed juice produced tuberculosis in one or two of the guinea-pigs when injected into the peritoneum. According to Bang, observations made in slaughter-houses show that in the immense majority of tuberculous cattle the lesions are so circumscribed, that under the now generally recognised rules of meat-inspection, their flesh may safely be passed for human consumption. According to Siedamgrotzky, in Germany, at the outside only from 2 to 3 per cent. of tuberculous animals are totally condemned, while 5 to 6 per cent.

are sent to the "Freibank," and about 92 per cent. are passed as quite wholesome.

Tuberculosis of the Udder.

In a recent article on Veterinary Dairy Inspection, Malcolm* states that one or more quarters of the gland may be infected, a posterior one being most frequently involved, although by no means invariably so. The disease usually commences as a slow, indolent swelling in the upper portion of the gland, and gradually increases, invading the whole quarter, sometimes affecting the entire gland. The enlarged gland is somewhat hard and fleshy to the feel; there is little or no pain, heat, or signs of an acute character, and the inguinal lymphatics are enlarged. In other forms of mastitis, except that due to the Actinomyces, the gland usually atrophies, while in tubercular mastitis a permanent increase in the size and density of the organ is an invariable result. It is generally admitted that the milk of tuberculous cows is much more liable to produce human tuberculosis than their flesh. This is proved by the fact that abdominal tuberculosis is practically confined to children under five years of age whose food consists very largely of raw milk; whilst the incidence is greatest in cases of hand-fed infants. Nothing except milk-infection can account for the prevalence among children of tabes mesenterica and allied tubercular disease. Nocard states that in his experience he never found the milk virulent when the udder was free from tuberculous lesions, and out of fifty-four cows seized for general tuberculosis which he specially examined, only three had tubercular lesions in the udder. Of the cows examined in 1898 in Edinburgh, 18.5 per cent. had tubercular udders. At Copenhagen the proportion is still lower. Bang estimates it at less than 3 per cent. of the number of tuberculous cows. Ernst and other observers have shown that tubercle bacilli may be present in the milk of cows with apparently healthy udders. They are, however, much less numerous than in cases where the udders are diseased. However, the difficulty of deciding as to the non-existence of tubercle in the mammary gland justifies the milk from suspicious cases being excluded from consumption. (For the special methods of examining and staining tubercle bacilli in milk, see Technique, p. 25.)

Prevention.—The principal thing, according to Bang, in combating bovine tuberculosis, is the prevention of infection by separating the

* Malcolm, *Jour. of Comp. Path. and Therap.*, vol. xiv., part i., p. 32.

sound from the diseased animals. This separation is accomplished by means of the tuberculin test, and the animals in which no reaction takes place are, at least to the extent of 90 per cent., free from tuberculosis.

The progeny of bulls and cows suffering from advanced tuberculosis must not be reared, but all others may be kept, provided they are removed from the infected building, and, to prevent abdominal infection, fed after the first day with milk which has been boiled or heated to 85° C. During the first day they must be fed with colostrum milk.

Tuberculosis is occasionally met with amongst sheep, and every year in the large German slaughter-houses undoubted cases are recorded. MacFadyean* was the first to record an authentic case in this country.

The question of living tubercle bacilli existing in ordinary market butter has led to considerable investigation since Obermüller stated that in fourteen samples of butter he found genuine tubercle bacilli, capable of causing infection.

Rabinowitsch† in 1899 examined fifteen samples of butter obtained from fourteen different sources, and from two of this number derived from the same source, virulent tubercle bacilli were isolated, and some of the remaining thirteen produced pseudo-tuberculosis. No mixed infection was observed. In a later experiment genuine tubercle bacilli were found present in 77 per cent. of the samples examined.

Petri's results occupy an intermediate position between those of Obermüller and Rabinowitsch. He found genuine tubercle bacilli in 30 per cent., and acid-resisting bacilli in 60 per cent. of the samples examined. Horman and Morgenroth found tubercle bacilli in three out of ten samples of market butter examined (see Fig. 69). In some of the samples they found Rabinowitsch's acid-resisting bacilli; and in one of the experiments the resisting bacteria and genuine tubercle bacilli were found together. Glycerine-agar was of no use as a culture medium; blood-serum, with the addition of 5 per cent. of glycerine, being found the best medium in these investigations.

In Denmark since the 1st of June 1899 the cream is heated to 85° C. before the butter is made. The heating process has no injurious

* *Journal of Comp. Path. and Therap.*, vol. xiii., part i., p. 59.

† *Deutsche Med. Wochenschrift*, 1899, No. 1.

effect on the butter, but renders it innocuous. (See p. 26 for method of detecting tubercle bacilli in butter).

The acid-resisting tubercle-like bacilli found in butter are described by Horman and Morgenroth as follows:—

Microscopical Appearances.—Slender rods, similar to *Bacillus tuberculosis* in form, slightly bent, and sometimes thickened at the ends.

Motility.—Non-motile.

Staining Reactions.—When stained by Günther's method for tubercle bacilli, they are not decolorized, but their resistance is not so pronounced as with tubercle bacilli, as on the edges of the cover-glass and contact specimen of colonies many of the rods are stained slightly blue. In old cultures unstained portions in the stained rods were often observed. The reaction with the Gram method is positive.

Biological Characters.—*On Gelatine Plates* the growth is very slow. The deep-lying colonies are round, glistening, of a light-yellow colour, and finely granular throughout. The superficial colonies are also round, transparent, and possess irregular, finely serrated margins. The gelatine is not liquefied.

In Gelatine Stab-Cultures, growth occurs along the track of the needle, and after a long time a thick coating develops on the surface.

On slanted Agar, in twenty-four hours at 37° C., a white, creamy coating develops on the surface of the water of condensation, and a thin membrane is formed which extends on to the walls of the tube. In old cultures the growth is frequently very much corrugated, and sometimes also exhibits a yellow, orange, or light-brown colour.

Bouillon or *Glycerine-Bouillon* becomes clouded with a sediment, and in forty-eight hours a membrane forms on the surface, which sinks very readily to the bottom if the bouillon is shaken, after which another membrane forms, which extends up the sides of the tube. A slight formation of *indol* was observed in bouillon cultures.

On Potatoes the growth is rapid and plentiful, exhibiting a thick, greyish, moist coating in twenty-four hours.

Milk is not altered by the growth which gives rise to a yellowish-white membrane on the surface.

The characters above mentioned correspond to those of the acid-resisting bacteria discovered by Rabinowitsch.

Pathogenesis.—These acid-resisting bacteria produced disease-processes in guinea-pigs when injected into the peritoneum. The liver was the only organ in which the lesions bore a distinct resemblance to those of tuberculosis. The tubercles were of a greyish-white colour, and penetrated from the surface of the liver into the parenchyma, being easily detached from the surrounding tissue. Small yellow foci, up to the size of a pin-head, were also observed on the surface as well as in sections of the organs. The spleen, on the contrary, never exhibited the appearance of a genuine tuberculous spleen. The enlargement, dark colour, and characteristic marbling were wanting. In most cases extensive peritoneal adhesions were present. The mesenteric lymph-glands were not much enlarged. In one case softening was observed; it was of a purulent

nature, not caseous. These bacteria frequently cause peritoneal lesions which exhibit a decided tendency to heal. In cases where reparative changes were observed, the acid-resisting bacteria appeared to die and disappear, because in cover-glass specimens and sections from some of the guinea-pigs experimented with, the bacteria could no longer be detected. Two chickens and one dog injected intraperitoneally with a pure culture remained unaffected. Injection into the anterior chamber of the rabbit's eye caused inflammatory changes which did not persist very long. According to Rabinowitsch,* on the whole, it is not so virulent as Koch's bacillus, even with guinea-pigs. White rabbits and white mice are immune. Pure cultures are not so virulent as the original samples from which they are obtained. The nodules produced by the organism in the inoculated animals consist of accumulations of lymphoid elements associated with epithelioid and multinucleated cells, the bacilli lie towards the centre, and the process is exudative rather than proliferative in character. Typical giant cells, "nests" of epithelioid cells, and "typical tuberculous caseation are absent," and it is this that constitutes the main differential character between this affection and true tuberculosis.

Equine Tuberculosis.

In the horse this disease manifests itself in two forms:—

1. *The Abdominal Form*, which seems to be the more frequent, is characterised by confluent lesions in the spleen, sub-lumbar and mesenteric glands (for section of same, see Fig. 66), liver, and intestinal mucous membrane. According to Nocard, infection seems to take place by way of the alimentary canal. When the lungs are invaded as a sequel of the abdominal form, the fresh lesions are seen to consist of diffuse infiltration of the interlobular connective tissue without apparent tubercles, cavities, or centres of softening. According to the above authority, this would account for the absence of cough and discharge, or expectoration, and the non-transmission of the disease to other horses in the same stable. Nocard also states that in advanced cases an abundant polyuria, lasting for several weeks, occurs. Very long tubercle bacilli are extremely abundant in the lesions. Nocard considers that the bacillus of equine abdominal tuberculosis is more closely allied to the bacillus of avian tuberculosis than to the bacillus of mammalian tuberculosis.

2. *The Thoracic Form*.—In this type the disease seems to originate primarily in the lungs, because these organs and the bronchial glands are most severely affected. A genuine acute miliary tuberculosis sometimes occurs; at other times the parenchyma of the lungs is studded with small abscesses with a fibrous capsule, enclosing pus

* *Zeitschrift für Hygiene und Infektionskr.*, xxvi., p. 90.

that is very rich in bacilli. Nocard claims that the distinction drawn between the two types of equine tuberculosis, on clinical and pathological grounds, is confirmed by the determination of the causal agent, for while the thoracic form is referable to a type of bacillus corresponding to that of human tuberculosis, the abdominal form, as already mentioned, is due to a bacillus which is more closely allied to the bacillus of avian tuberculosis.

According to Galtier,* the ass, although offering a marked resistance to tuberculosis, is not absolutely refractory. It may contract the disease naturally, and it may be experimentally infected.

Canine Tuberculosis.

In the dog the disease also occurs in two forms, the *abdominal* and the *thoracic*. Many cases have been recorded, the principal lesions mentioned occurring in the liver, hepatic and mesenteric glands. Generalized tuberculosis of both lungs and of the bronchial glands has also been observed.

Tuberculosis of Swine.

The pig is very susceptible to the experimental disease, and according to some authors, the scrofulous conditions (see Fig. 68) occasionally observed in pigs are due to tuberculosis. According to Nocard, the disease in the pig often develops with great rapidity and passes unperceived. In the chronic form the bacilli are rare, and appear to have partly lost their virulence; for when inoculated into guinea-pigs they produce chronic disease, whereas the period of incubation becomes shortened when the bacilli are inoculated through a series of guinea-pigs.

According to Nocard, nine out of every ten pigs are infected through the alimentary canal. Many investigators have produced the disease by feeding pigs with milk from cows with tuberculous udders. In 1897, pigs were kept under the shambles in San Francisco, and fed on the offal which fell down a shoot. The percentage of tuberculosis that occurred in these swine was beyond comprehension, the liver and spleen being studded with masses of tuberculous nodules.

Tuberculin.—The use of tuberculin as a *curative* agent has not

* *Journal de Méd. Vét.*, Feb. 1900. Ref., *Journ. of Comp. Path. and Therap.*, vol. xiii., part i., p. 76.

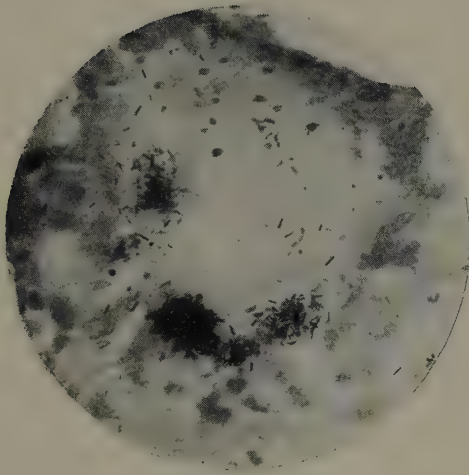


FIG. 67.—*B. tuberculosis*. Section of tubercle from spleen of pig.
Ehrlich's stain. $\times 600$.

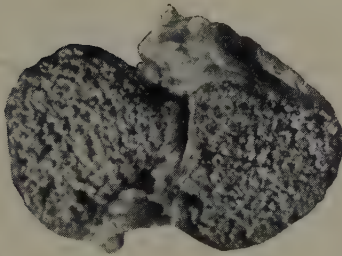


FIG. 68.—Scrofulous gland from neck of pig which
has been cut in two, and is filled with caseous
and calcareous matter.

fulfilled the expectations at first anticipated. When injected into healthy persons it induces no reaction, but in tubercular patients a pronounced systemic reaction results. Koch has lately produced a new tuberculin, known as TO. and TR., prepared by triturating dried cultures of tubercle bacilli in a mortar. The mass is then mixed with distilled water and placed in a centrifugal machine, when an opalescent, transparent, whitish fluid is obtained free from bacilli. Trudeau and Baldwin have recently conducted experiments with this new preparation in New York, and found that it still contained living tubercle bacilli, capable of producing tuberculosis in guinea-pigs; results since confirmed by other investigators. We may conclude from this that this preparation may be a considerable source of danger if carelessly prepared. According to Koch's original method, the purity of six to eight weeks' old veal bouillon cultures of the tubercle bacilli were first tested by microscopical examination, after which they were poured into a suitable vessel, and evaporated to one-tenth the original volume over a water-bath. The liquid was then filtered through porcelain. The crude tuberculin obtained by this process contains 40 to 50 per cent. of glycerine. It is soluble in water, insoluble in alcohol, passes readily through dialyzing membranes, is not destroyed by boiling, and preserves its activity indefinitely.

Tuberculin is very valuable as a *diagnostic* agent in bovine tuberculosis. For this purpose it is diluted with nine parts of water containing a half per cent. of carbolic acid. About 3 c.c., or 60 minims, are injected subcutaneously; the point of injection is immaterial, but the side of the neck where the skin is thin is the most suitable place. It is also more practical to use a large-sized inoculating needle instead of small needles. The hair is clipped from the part selected, which is thoroughly cleansed and disinfected before the injection is made. Before the tuberculin test is applied, the temperature of the animals ought to be taken every two hours, at least six or seven times before the injections are made, as in many animals the variations are sufficiently constant to make their determination by precise measurements practically necessary in every tuberculin test from which reliable results are expected. After the injection is made, the temperature ought to be taken again in eight to ten hours, and from then on every two hours, until a decided reaction, continuous during several hours, has occurred, or until eighteen to twenty hours have elapsed since the time of

injection. The febrile reaction following the subcutaneous injection of tuberculin in tuberculous cattle begins six to ten hours after the injection. It reaches the maximum in nine to fifteen hours, the temperature falling to normal in eighteen to twenty-six hours after the injection. The elevation of temperature sufficient to constitute a "reaction" has variously been given at 0.5° to 1° C., but consideration of the number of degrees the temperature after the injection rises above the temperature before the injection is not alone sufficient—the *duration* of the reaction must also be taken into account. Advanced cases of tuberculosis occasionally fail to react, while the reaction is frequently greatest in young animals in the first stages of the disease. In fact, the normal variation of the temperature of an animal during the course of the day is frequently so great, that if the variation is not determined, and the temperature is taken only once previous to a tuberculin injection, it is merely a matter of chance if a high temperature, natural to the animal and independent of the action of the tuberculin injection, is not confounded with and erroneously taken for a tuberculin reaction. It is necessary in testing cattle that have changed hands to give them a few days' rest, and to use a larger dose than usual, or otherwise the temperature may not rise to the usual height. The range of the thermal reaction gives no indication of the extent of the tubercular lesions in an animal. Instances are recorded where a second injection of tuberculin has altogether failed to produce a reaction in animals which gave a very decided reaction after the first injection, notwithstanding that the two injections were separated by a very considerable period of time. This question of the non-reaction to a second injection is a matter for future investigation. Should the foregoing instances be correct, there is nothing to hinder unscrupulous persons to so prepare their animals, that when submitted to a *tuberculin* test the results are negative.

Bang (Vet. Congress, 1899) finds that animals through repeated inoculations acquire a tolerance for tuberculin. It is true this is comparatively seldom observed after the first reaction, but by repeating the injection several times, one can, as a rule, bring about a temporary insusceptibility. The fact that when the tuberculin test is repeated after the lapse of a year, a considerable number of animals that reacted on the first occasion fail to do so on the second, is more difficult to understand than the immunity acquired by repeated injections at short intervals. Bang states he found comparatively slight lesions in many

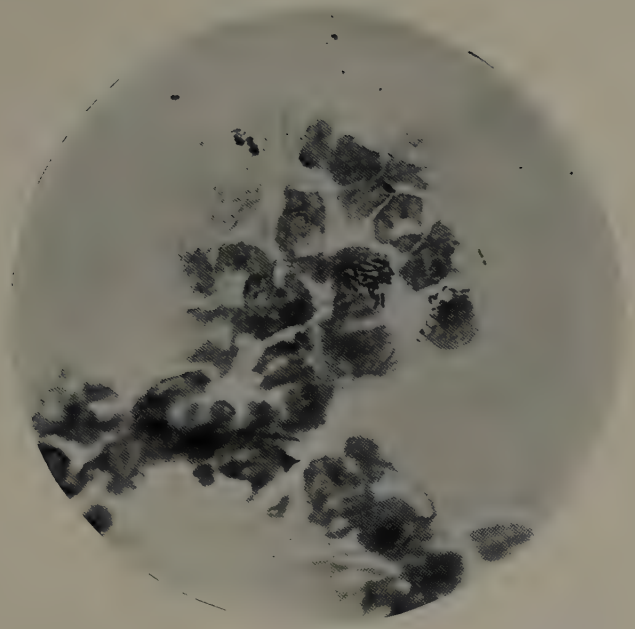


FIG. 69.—*B. tuberculosis*. Smear preparation from guinea-pig inoculated with culture from guinea-pig inoculated with butter. $\times 1000$.

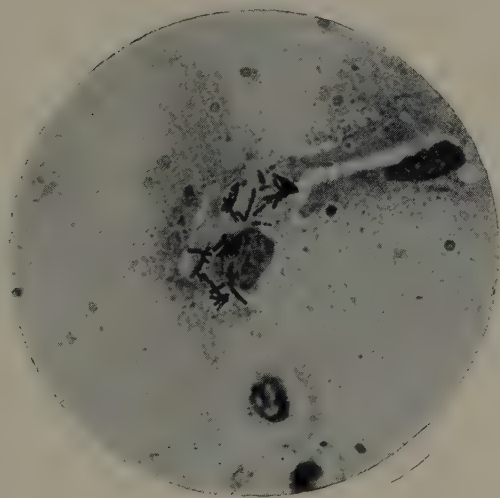


FIG. 70.—*B. tuberculosis* (avian). "Branched forms." Cover-glass specimen from the liver of a chicken. Ehrlich's stain. $\times 1000$.

of these cases on post-mortem examination, but in other cases extensive disease was observed. Bang, therefore, warns owners in the strongest possible way against "regarding such animals as cured."

In a recent Report* by a Committee of the Royal Agricultural Society of England on the reliability of the tuberculin test, it is shown that tuberculin falls short of infallibility in so much that there is in every case a period after infection during which it provokes no reaction. Recent experiments in England and France to determine the exact period of non-reaction show, that in the French experiments forty-eight days, and in the English experiments fifty-one days, was the maximum period elapsing after infection, before the animal acquired the property of reacting to tuberculin. Non-reaction unfortunately, as MacFadyean† states, "falls a good way short of being absolute proof of freedom from tuberculosis, but in the present state of knowledge it is by far the most conclusive evidence of that kind that is obtainable while the animal is still alive. In like manner, not every animal in which the temperature rises after an injection of tuberculin is tuberculous, but the exceptions are not sufficiently numerous to seriously weaken the weight of a reaction as evidence of infection."

Avian Tuberculosis.

The bacillus of avian tuberculosis is but a variety of the bacillus of mammalian tuberculosis. This has been incontestably proved by Nocard, as we shall see below.

Microscopical Appearances.—It is thinner and more slender than the bacillus found in man and mammals, club and branched forms being more frequent (see Fig. 70).

Staining Reactions.—It is more readily stained than the mammalian bacillus, but exhibits a similar reaction towards decolorizing agents.

Biological Characters.—It is not so difficult to cultivate as the other forms, growing in ordinary agar and bouillon; but the addition of glycerine to the media assists the growth to a great extent. The avian variety grows more rapidly than the bacillus of mammalian tuberculosis. The cultures are not so dry, but much moister, and form a coherent coating. On solid culture media, the growth forms

* *Journ. of Comp. Path. and Therap.*, vol. xiii., part iv., p. 368.

† *Ibid.*, vol. xiv., part i., p. 73.

a film over the water of condensation. Old cultures exhibit a yellowish colour.

The bacillus of avian tuberculosis grows just as luxuriantly at 42° to 45° C. as at 37° C., a characteristic not exhibited by the mammalian variety, which ceases to develop at such high temperatures. The avian bacilli have doubtless become adapted to a high temperature during their sojourn in the body of the bird, the normal temperature of birds being 41° to 42° C. The avian bacilli are more resistant towards heat than the bacilli of human tuberculosis, being only killed by exposure for fifteen minutes at 70° C.

The bacilli are found in the tuberculous lesions, which are characterized by tough masses of nodules, often accompanied by calcareous deposits. Giant cells are very scarce. A few cases of tuberculosis, of avian type, have been observed in man and mammals.

Pathogenesis.—Most birds are very susceptible, and can be infected by all the different methods of infection. Guinea-pigs and dogs are somewhat refractory, without, however, possessing a perfect immunity. The avian bacilli usually develop badly in mammals, and mammalian bacilli are acclimated with difficulty in birds.

Nocard found that fowls could be infected by inoculation with human or bovine tuberculous matter. On the other hand, the dog and guinea-pig, although most susceptible to the action of both human and bovine tuberculosis, are very refractory to avian tuberculosis. If, however, a guinea-pig is inoculated intraperitoneally with avian tuberculous material, it often dies, showing a special kind of lesion. Nocard placed a glycerine-bouillon culture of the bacilli of human tuberculosis in little sacs of collodion, and introduced them into the body cavity of chickens. The sacs were removed in from five to eight months, and found to contain a sort of paste made up of bacilli. Cultures prepared from this paste grew extremely well, and the interesting fact was observed that the bacillus had lost the characters of the germ originally introduced, and had assumed those of the avian bacillus. The bacillus now grew at high temperatures, and the cultures resembled those of the avian bacillus. The bacillus was not virulent enough to produce tuberculosis in fowls until it was passed through two or three fowls, and a period of four to six months had elapsed. In one case a fowl suddenly contracted tuberculosis eleven months after the introduction of the collodion sac. At the post-mortem examination, it was found that the sac had burst,

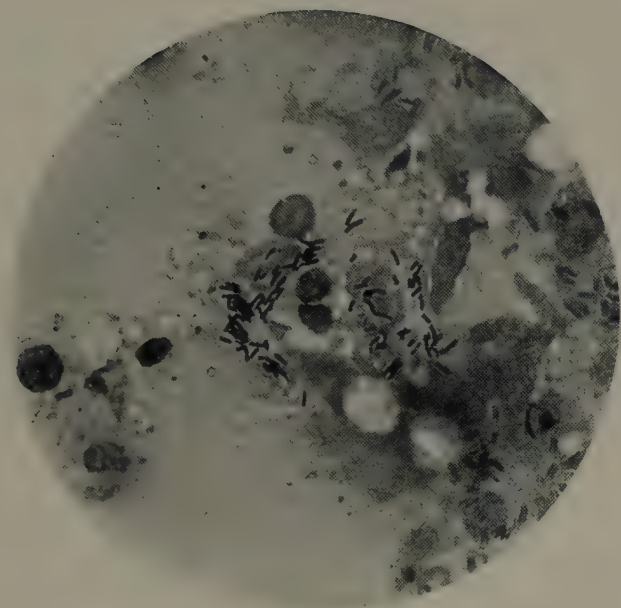


FIG. 71.—*B. pseudo-tuberculosis*. Smear preparation from nodule in guinea-pig inoculated with butter. $\times 1000$.

T. Bowhill, F.R.C.V.S., Photo.]

[Face page 186.

showing that development had proceeded far enough when the sac ruptured to produce tuberculosis in the fowl. Nocard concluded that human and avian tuberculosis are only two varieties of the same disease. It appears probable from his investigations that the human subject may at times contract tuberculosis from the fowl.

PSEUDO-TUBERCULOSIS.

The term pseudo-tuberculosis is applied to certain pathological processes which resemble genuine tuberculosis, but are dependent on other causes than *B. tuberculosis*. The etiology of pseudo-tuberculosis is remarkably manifold. It may be produced by inanimate foreign bodies, animal parasites, bacteria, or by vegetable parasites. The pseudo-tuberculoses caused by foreign bodies can be produced easily with a variety of substances, *but are not transmissible from animal to animal*.

The pseudo-tuberculosis produced by animal parasites is only found in animals. In the cat it is caused by the *Ollulanus tricuspis*; in the sheep, by the *Pseudalius ovis pulmonalis*; in the calf, by the *Strongylus refulgens*; in the dog, by the *Strongylus vasorum*.

Miura records a case in a man who died of beri-beri, a fibrous tubercle caused by distoma eggs being found in the mesentery.

Many cases of pseudo-tuberculosis in animals caused by bacteria are mentioned, the principal being zoöglæic tuberculosis, described by Malassez and Vignal. The following is a description of the organism they found:—

Microscopical Appearances.—Thick, short rods, frequently coccoid, in the form of chains in small groups, or in zoöglæa. Spore-formation absent. (See Fig. 71.)

Staining Reactions.—The reaction with the Gram method is negative. Sections are best stained with Malassez's blue prepared as follows:—

Two per cent. solution of sodium carbonate,	10 c.c.
Anilin-water,	5 c.c.
Absolute alcohol,	3 c.c.
Solution made with 9 vols. of distilled water and 1 vol. of concentrated solution of methylene- blue in 90 per cent. alcohol,	3 c.c.

Sections remain in this stain for two to three days; they are then

washed in water, stained with methylene-blue, and cleared in oil of Bergamot or turpentine.

Biological Characters.—*On Gelatine Plates* non-liquefying colonies develop somewhat similar to those of *B. typhi abdominalis*.

In Gelatine Stab-Cultures the growth resembles a flat-headed nail.

On Agar, a greyish growth, giving off a fœtid odour.

On Potatoes, a yellowish coating.

In Bouillon, a flaky cloudiness occurs at first, then a sediment is formed, the upper portions of the medium becoming clear.

Pathogenesis.—The bacillus of pseudo-tuberculosis is pathogenic for guinea-pigs (death occurring in five to six days), also for dogs and horses. The post-mortem lesions, especially in the abdominal organs, resemble those of genuine tuberculosis, these organs being most affected. The differential diagnosis is not difficult, the pseudo-tuberculosis bacillus being readily stained, and yielding characteristic cultures having a rapid growth.

Courmont also describes a bacillus found in pseudo-tubercular pleural lesions in the ox. This bacillus is short, with its substance condensed at both ends, and a clear, slightly constricted middle; it does not form chains nor diplococci. It is both aerobic and anaerobic, grows quickly, is easily cultivated in all kinds of media up to 46° C. It is pathogenic for guinea-pigs, which die in four to eight days with generalized pseudo-tuberculosis. In rabbits it produces disseminated and confluent tubercles in the spleen, liver, and lungs. The bacillus is also found in the blood of inoculated animals, and though it may become disseminated throughout the body, it does not affect the lymphatic glands.

The pseudo-tuberculosis caused by the higher organized vegetable parasites likewise manifests itself, especially in animals. Different forms of *Streptothrices* and *Aspergilli*, particularly the *Aspergillus glaucus* and *fumigatus*, require consideration. Pigeons often succumb from a miliary "tuberculosis," *A. fumigatus* being found present in the interior of the granulations. Lung-affections are also sometimes observed in individuals engaged in the feeding of pigeons, the disease being apparently due to the same parasite, *A. fumigatus* being found in the expectoration of the patients. It is probable that the parasite is conveyed with the grain used to feed the pigeons. Eppinger records a case of pseudo-tuberculosis in man due to the *Streptothrix eppingeri*.

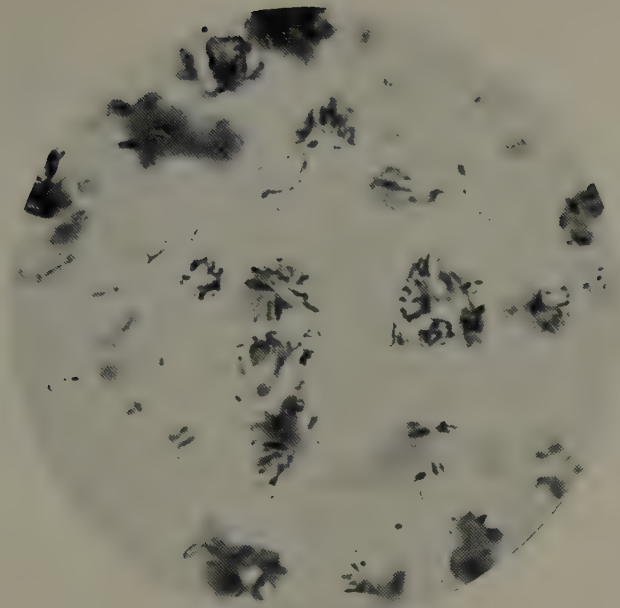


FIG. 72.—*B. leproe*. Section of cutaneous nodule. Stained by Claudius method. $\times 1000$.

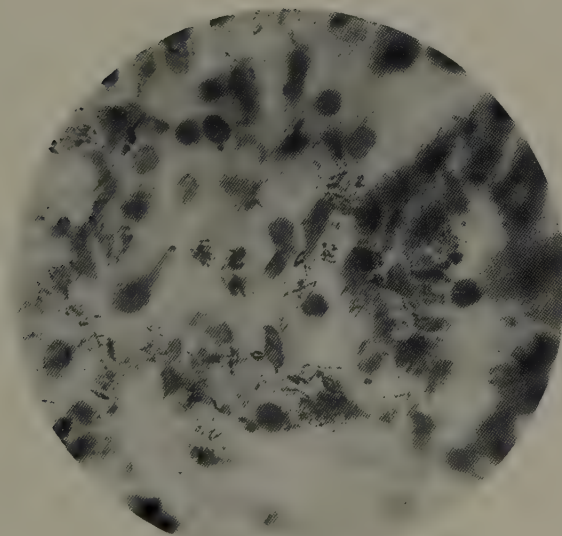


FIG. 73.—*B. leproe*. Section of affected tissue.

Valée (*Recueil de Méd. Vétér.*) has described a form of pseudo-tuberculosis in calves due to an organism which is much smaller than that of swine-erysipelas. The germ occurs either isolated or in small masses in the diseased tissues, and stains by the Gram-Nicolle method. When isolated, and cultivated on different media, it produces the original lesions in various experimental animals. In affected calves the principal lesions are found in the liver, which, though normal in size, is covered with fine granulations or tubercles (in some cases confluent) of a greyish colour. A slight perihepatitis was observed, and the tissue of the gland was very friable.

Flexner * (1897) found an organism which he named *Streptothrix pseudo-tuberculosis* in a case of pseudo-tuberculosis in man. The lesions in the lungs consisted of consolidation, necrosis, and the early formation of cavities. At autopsy, nodules similar to those produced by *B. tuberculosis* were observed in the peritoneum, omentum, liver, and spleen. It somewhat resembles a *Streptothrix* found by Buchholtz † (1897) in a man who died from a similar affection. Neither Flexner nor Buchholtz succeeded in cultivating the organisms they found.

BACILLUS LEPRÆ.

This organism, discovered by Hansen in 1879, is found chiefly in the interior of the peculiar round and oval cells found in cutaneous leprous tubercles. The bacilli have also been found in the lymphatic glands, liver, spleen, testicles, and in the thickened portions of involved nerves in the anæsthetic form of the disease. They have also been found occasionally in the blood. The bacilli lie in the leprous cells in great numbers, and also in the lymph-spaces outside of these cells. They are not found in the epidermal layers of the skin, but, according to Babes, they may penetrate into the hair follicles.

Microscopical Appearances.—The bacillus resembles the tubercle bacillus in form, but is more uniform in length and not so frequently bent or curved. It measures 4 to 6 μ in length, and less than 1 μ in width. The ends of the rods are pointed, and in stained specimens unstained spaces similar to those in the *B. tuberculosis* are present.

Motility.—Non-motile.

Staining Reactions.—The bacilli stain readily with the anilin

* *Journ. of Exper. Med.*, vol. iii., p. 435.

† *Zeitschr. f. Hyg.*, vol. xxiv., p. 470.

dyes, also by the Gram and Claudius methods (see Figs. 72 and 73). For differential staining reactions, see under Tuberculosis, p. 172.

Biological Characters.—It has not yet been cultivated, but on account of its constant presence in leprous tissues, there can be no doubt as to its being the cause of the disease.

PSEUDO-TUBERCULOSIS OVIS.

History.—This disease has been observed in thousands of sheep in Australia, being first observed at the Melbourne Abattoir.

The lymphatic glands were found affected with nodules in 15 to 70 per cent. of the animals; and on an average each sheep has two to three glands affected. The health and general condition of the animals does not seem to suffer. The glands affected are chiefly the prescapular and superficial inguinal. One or two nodules were sometimes found in the kidney, but never in the liver or mesentery. The affected glands are enlarged, and feel like a sac containing fluid, the capsule being firm and thick.

Morphology of the Specific Germ.—Small non-motile oval bacilli 1.5 to 2 μ long and 0.8 μ broad. They stain easily with the ordinary anilin dyes and by the Gram method.

Growth.—The best growth takes place on agar and blood-serum at 37° C.

Animals affected.—Guinea-pigs inoculated with large doses die in twenty-four hours, an extensive local œdema being present. Small doses kill in four to seven days, caseous nodules being formed in the subcutaneous tissue, the adjacent lymph-glands, and in the internal organs. Sheep are more susceptible than guinea-pigs. Inoculations with pure cultures produce changes indistinguishable from those found in the naturally acquired disease. A pure culture may be recovered from the experimentally inoculated animals. Preisz found an organism very similar to this in the kidney of a lamb, and called the disease "Pseudo-tuberculosis ovis." Other outbreaks of a similar disease are mentioned by Stending, Turski, and Baumgarten.

BACILLUS SMEGMATIS.

Found in the smegma præputii and in smegma from between the scrotum and thigh, or between the labiæ. It is also found in the cerumen, and occasionally on the skin.

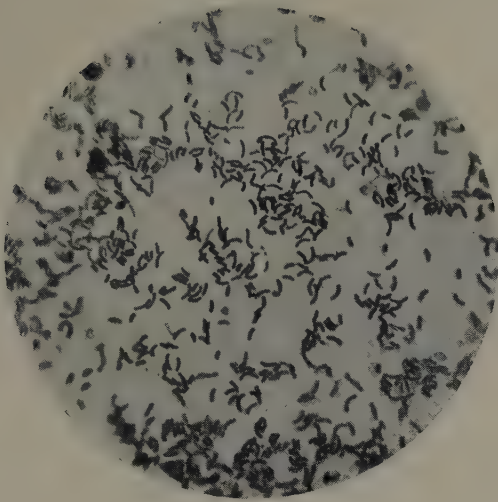


FIG. 74.—*Spirillum cholerae asiaticae*. Agar culture. Fuchsin. $\times 1000$.



FIG. 75.—*Spirillum cholerae asiaticae*, with flagella. Stained by Bowhill's method. $\times 1000$.

Microscopical Appearances.—The bacilli lie in clusters either in or between the epithelial cells, being very similar in size and form to *B. tuberculosis*.

Staining Reactions.—They stain with difficulty, are acid-resisting when stained by the methods for tubercle bacilli (see p. 172), but are decolorized when treated one minute with absolute alcohol. The reaction with the Gram method is positive.

Biological Characters.—Doutrelepoint and Matterstock obtained a culture of a similar organism on coagulated hydrocele-fluid.

Differential Diagnosis (see p. 172).—It is most likely to be mistaken for the *B. tuberculosis* in the examination of urine.

SPIRILLUM CHOLERÆ ASIATICÆ.

Discovered in 1883 by Koch in India in all cases of Asiatic cholera.

Microscopical Appearances.—Slightly curved rods with rounded ends from 0.8 to 2 μ in length, and about 0.3 to 0.4 μ in breadth. The rods are usually curved like a comma, but are occasionally in the form of a half circle, or when two rods remain connected and are curved in opposite directions they may form an S-shaped figure. The typical comma shape is best observed in specimens prepared from young cultures (see Fig. 74). When the spirilla remain attached together, they form spirals of varying length. This condition is frequently observed in old cultures or on the addition of weak antiseptics, *i.e.*, alcohol, to the medium. Chains of spirilla are very seldom observed in cholera dejections. These chains are especially frequent in the peritoneal exudate of inoculated guinea-pigs.

Motility.—Actively motile. They possess a single terminal flagellum. (See Fig. 75, showing flagella stained by the author's orceïn method.)

Spore-Formation.—Unknown.

Staining Reactions.—The best results are obtained with a saturated watery solution of fuchsin, or with carbol-fuchsin. The stain must be allowed to work a considerable time. The reaction with Gram's method is negative. Sections may be stained with Löffler's methylene-blue solution.

Biological Characters.—The cholera germ grows on all the ordinary nutrient media under aërobic conditions. The culture media

must possess a decided alkaline reaction, as the germ is very sensitive to the smallest quantity of acid. The minimum temperature at which development takes place is 8°C ., the optimum lies between 30° and 40°C .

On Gelatine Plates the development takes place best at 22°C . In twenty-four to thirty hours, when examined under a low power, small yellowish-white granular colonies with uneven rough edges are present. The surface of the young colony looks as if it were covered with little fragments of broken glass. As the colony grows older an ill-defined halo is seen to surround it, and it exhibits a peculiar roseate hue by transmitted light. Later, as liquefaction proceeds, the colony loses the powdered glass appearance and forms an irregular brownish-white mass surrounded by a zone of liquefaction.

In Gelatine Stab-Cultures development occurs along the line of the stab, the liquefaction beginning at the surface. On the second day, at 22°C ., a short funnel is formed with a very narrow mouth, the upper portion of which contains air, and below this a whitish viscid mass. The funnel now increases in depth and diameter, and in four to six days may reach the edge of the test-tube; in eight to fourteen days the upper two-thirds of the gelatine is liquefied, and subsequently the gelatine is completely liquefied.

On Agar Plates the growth is not so characteristic as on gelatine. The surface colonies exhibit a peculiar light greyish-brown, transparent appearance.

On Agar Stroke Cultures a greyish-white, moist, shining coating develops.

Blood-Serum is liquefied slowly.

On Potatoes, at 37°C ., a thin, semi-transparent, brown or greyish-brown layer is developed. On some potatoes no growth takes place, but development takes place if the potatoes are rendered alkaline with a solution of soda, or cooked in a 3 per cent. solution of common salt.

Milk is a favourable culture medium.

Bouillon is clouded, and in the majority of cases, when placed in the incubator, a thin membrane forms on the surface of the media.

It also develops in 1 per cent. watery solution of peptone, to which $\frac{1}{2}$ per cent. of sodium chloride has been added. If the peptone used is not alkaline, then the medium must be rendered alkaline with a solution of soda.

Indol Reaction.—When a drop of pure hydrochloric or sulphuric acid is added to cholera cultures grown in peptone media, a rose or purple-red colour results, which is known as the nitroso-indol reaction. The germ possesses the faculty of first forming indol, and then changing the traces of nitrates in the culture solution into nitrites. Other vibrios besides that of cholera exhibit the nitroso-indol reaction, *i.e.*, *V. Metschnikoff* and *V. Berolinesis* (see later). Finkler and Prior's vibrio and Denecke's cheese vibrio also form indol, but no nitrite, the addition of a pure acid that does not contain nitrous acid producing no red colour. In bouillon, under certain circumstances, the reaction fails when either too much or too little nitrates are present.

There are two methods whereby genuine cholera germs are differentiated from other similar vibrios, known as Pfeiffer and Gruber's reactions. These reactions are specific.

(1.) *Pfeiffer's Reaction* is produced as follows :—Some blood-serum of a guinea-pig or other animal rendered immune to cholera is diluted with ordinary bouillon in the proportion of 1 to 100. To 1 c.c. of the diluted serum, a platinum loop (capable of holding about 2 mg.), full of a bouillon culture of the organism under investigation, is added, after which the mixture is injected into the peritoneal cavity of a guinea-pig weighing about 200 grams. Every five minutes some of the peritoneal effusion is removed by means of a fine glass capillary pipette, and examined, both stained and unstained. If they are genuine cholera germs they will at first be observed to become non-motile, and within about twenty minutes they become completely disintegrated. When the above phenomena are absent, then the vibrio belongs to another species. To prevent an error arising, a control guinea-pig is inoculated intraperitoneally with 1 c.c. of *normal* serum-bouillon mixture, 1 to 100, to which one loop of the suspected culture is added. If in twenty minutes the peritoneal effusion contains living motile vibrios (which were killed with immune serum within the body), then the diagnosis of Asiatic cholera can be given with safety.

(2.) *Gruber's Agglutination Reaction.*—A small portion of cholera culture is added to the serum (diluted 1 to 50, 1 to 100, etc.) of an animal immunified against cholera. The mixture is examined at once with a high power. If the vibrios become non-motile, collect together in masses (agglutinate), then the germs are without doubt genuine cholera vibrios.

Agglutination can also be observed macroscopically by inoculating bouillon with the suspected vibrio and adding cholera-immune serum in the same proportions as above. If in sixteen to twenty-four hours the vibrios have gathered together in flakes at the bottom of the tube and the upper portions of the fluid remains clear, it is the cholera spirillum.

Vitality.—The cholera germs do not exhibit much resistance. They are destroyed in four minutes in water heated to 52° C. They withstand lower temperatures better; in ice they lose their vitality in a few days. The addition of 0.07 to 0.08 per cent. hydrochloric or nitric acid to neutral culture-media prevents their growth; this explains why normal gastric juice, which contains about 0.2 per cent. of hydrochloric acid, destroys them. When spread in a thin layer, and dried, they die in three hours. In moist surroundings under favourable circumstances they live a long time—about nine months. They may survive for six months in agar and gelatine cultures. Weak solutions of the ordinary antiseptics kill them very quickly; a $\frac{1}{2}$ per cent. solution of carbolic acid kills them in a few minutes. They sometimes remain alive for weeks in dejections of cholera patients, but this occurs only under extremely favourable circumstances. They multiply to some extent in sterilized river or well-water, and may preserve their vitality in such water for several months. In milk or water containing other bacteria they die out in a few days. In greatly diluted bouillon-media they may outgrow common saprophytic bacteria, forming a film upon the surface of the medium.

When mixed with normal fæces the cholera spirilla survive but a few days.

Pathogenesis.—The introduction of the organisms into the human stomach may cause no bad effect; but sometimes a more or less intense diarrhoea results (self-infection by Pettenkoffer and Emmerich), and in other cases genuine dangerous cholera with all its clinical symptoms may supervene. A young doctor died in Hamburg of typical cholera, caused by a drop of peritoneal exudate containing vibrios getting into his mouth whilst testing for Pfeiffer's reaction. Subcutaneous injection in man only causes local symptoms and slight fever. According to Klemperer the blood thereby acquires immunizing properties.

A disease resembling cholera can be produced in guinea-pigs by direct introduction of the vibrio in the duodenum—evading the

stomach and tying the gall-duct—or by the introduction of the germ into the stomach, previously rendered alkaline with a solution of soda, at the same time injecting 2 to 3 c.c. of tincture of opium into the peritoneal cavity. Tying the gall-duct and injecting tincture of opium into the peritoneal cavity interrupts the peristaltic action of the bowels.

Metschnikoff has shown that normal suckling rabbits may be successfully infected by smearing the mother's teats with cholera cultures.

Bacteriological Diagnosis.—(1.) *Microscopical Examination.*—Cover-glass specimens are prepared from mucus flakes in the fæces, and are stained with a dilute solution of carbol-fuchsin. The diagnosis of Asiatic cholera is fairly certain when the individual vibrios appear to lie behind each other in one direction like a small swarm of fish in a stream. It is, nevertheless, desirable that cultures should be made.

(2.) *Examination by Cultures.*—A series of gelatine plate cultures are prepared from the fæces, when possible from a flake of mucus.

(3.) *Peptone-Water Culture-Method of Koch and Schottelius.*—Besides preparing plates, it may be necessary to resort to the use of peptone-water cultures, because in cases where the cholera germs are not numerous they do not develop, being outgrown by the ordinary fæces bacteria.

An *Erlenmeyer flask* containing 1 per cent. peptone and $\frac{1}{2}$ per cent. common salt solution is inoculated with a platinum loopful of suspected fæces or mucus and placed in the incubator at 37° C. As soon as the fluid exhibits the slightest trace of turbidity, which generally occurs in six to twelve hours, a portion is removed from the surface and examined in a *hanging-drop*, cover-glass specimens being also prepared. If a pure culture is obtained, then the diagnosis is assured. It is not always so simple, as the surface growth is sometimes contaminated with other bacteria, most frequently by *B. coli*, which renders it necessary to make plate cultures, the number of cholera germs having augmented by the preceding cultivation in the peptone solution.

Pure cultures are now obtained from the isolated colonies on the plate cultures, and tested for the nitroso-indol and Gruber and Pfeiffer reactions. Should these tests yield positive results, then the diagnosis is conclusive and certain. Agar plates can be used instead of gelatine

plates, and possess the advantage that they can be placed in the incubator at 37° C. and examined in eight to ten hours.

Examination of Water.—It is frequently necessary, to obtain satisfactory results, to use large quantities of suspected water. About 100 to 1000 c.c. of the suspected water is placed in sterile flasks, and to each sample 1 per cent. peptone (Witte's peptone is the best) and $\frac{1}{2}$ per cent. of common salt is added, the solution being rendered alkaline. The sterile peptone and salt solutions should be kept ready for use. Plates are prepared from the peptone solution after it has been incubated.

SPIRILLUM OF FINKLER AND PRIOR.

This organism was isolated from the dejections of patients with *cholera nostras* which had been allowed to stand for some days, but it has since been proven to possess no etiological significance in that disease.

Microscopical Appearances.—It bears a great resemblance to the cholera germ, but is somewhat longer and thicker, and not so uniform in diameter, being often thicker in the middle than at the poles.

Motility.—Actively motile. Possesses a single flagellum at one end. (See Fig. 76, stained by the author's orcein method.)

Staining Reactions.—Stains with the ordinary anilin dyes, best with an aqueous solution of fuchsin.

Biological Characters.—Aërobic and facultative anaërobic organism; grows at ordinary room-temperature.

On Gelatine Plates small, white, punctiform colonies develop in twenty-four hours, which on microscopic examination are seen to be finely granular and yellowish, or yellowish-brown in colour; the gelatine around the colonies liquefies rapidly, and when the organism is abundant, liquefaction is usually complete in twenty-four hours. Isolated colonies on the second day form saucer-shaped depressions in the gelatine.

In Stab-Cultures liquefaction progresses much more rapidly than with the cholera vibrio, a stocking-shaped pouch of liquefied gelatine appearing in two days, the whole media being liquefied in about a week; a whitish film forms on the surface of the liquefied media.

On Agar a moist shiny layer rapidly covers the entire surface.

On Blood-Serum the growth is rapid and causes liquefaction.

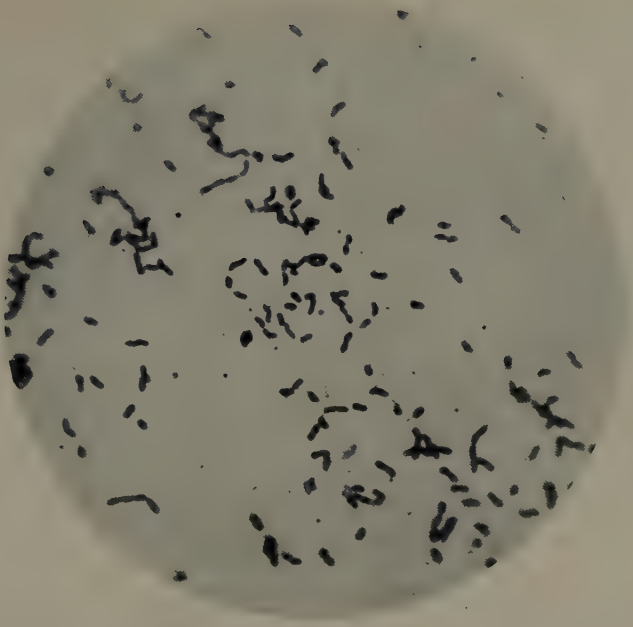


FIG. 76.—*Spirillum Finkler-Prior*, with flagella. Stained by Bowhill's method. $\times 1000$.

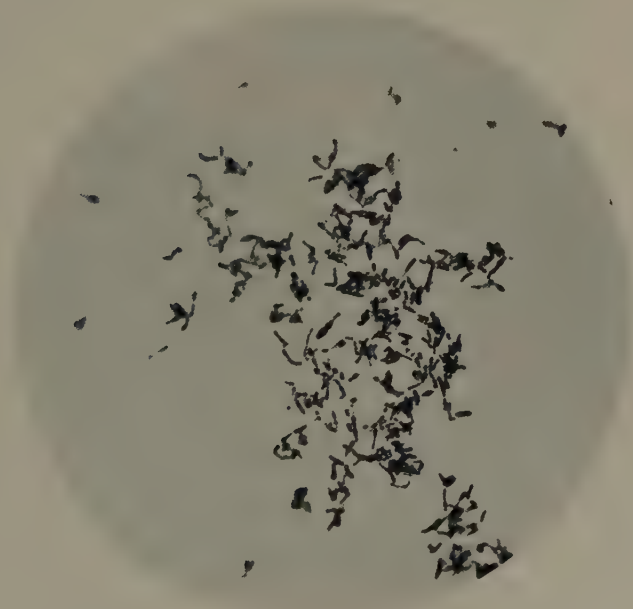


FIG. 77.—*B. diphtheriae*. Blood-serum-agar culture. Claudius stain. $\times 1000$.

On Potato at room-temperature a shiny, greyish-yellow, glistening layer is formed, which soon spreads over the surface. (*The cholera vibrio exhibits no growth on potato at room-temperature.*) The cultures give off a strong putrefactive odour, and in media containing sugar the germ produces an acid reaction.

Pathogenesis.—Pathogenic for guinea-pigs when injected into the stomach previously rendered alkaline with soda.

SPIRILLUM TYROGENUM (DENECKE).

This vibrio was obtained by Denecke from old cheese.

Microscopical Appearances.—Curved rods and long spiral filaments resembling the cholera vibrio, the diameter of the individual cells being uniform throughout, so that it more closely resembles the cholera vibrio than does that of Finkler and Prior.

Motility.—Actively motile, possessing a single flagellum at one end.

Staining Reactions.—Stains with the usual anilin dyes, best with an aqueous solution of fuchsin.

Biological Characters.—Aërobic and facultative anaërobic vibrio growing at ordinary room-temperature. It grows more rapidly than the cholera vibrio, but not so rapidly as the vibrio of Finkler and Prior.

On Gelatine Plates small punctiform colonies develop, which on the second day are about the size of a pin-head, and have a yellowish colour. Examined under a low power the colonies appear coarsely granular, of a yellowish-green colour in the centre, and paler towards the margins; funnel-shaped cavities are formed when liquefaction commences.

In Gelatine Stab-Cultures liquefaction takes place along the track of the needle, the vibrios sinking to the bottom in a mass, while a thin yellowish layer forms upon the surface; complete liquefaction takes place in about two weeks.

On Agar a yellowish-white coating is formed on the surface.

Blood-Serum is quickly liquefied.

In Bouillon or Peptone Solution the nitroso-indol reaction is wanting.

Pathogenesis.—Doubtful.

MILLER'S SPIRILLUM.

Obtained by Miller from a carious tooth. *Morphologically*, as also in its staining reactions, it is indistinguishable from the Spirillum of Finkler and Prior.

Motility.—Non-motile.

Biological Characters.—On *Gelatine Plates* small transparent pits of liquefaction appear in twenty-four hours; in the centre of the colony a minute white speck can be seen. Examined under a low power, the larger colonies are granular and regularly rounded, and usually surrounded by a peripheral zone somewhat darker than the central portion of the colony. On close examination the circumference can be observed to be fringed by short hair-like outgrowths usually twisted in all directions. *The deeper colonies* are round, sharply circumscribed, of a pale yellowish or greenish-yellow colour, and marked by delicate irregular lines or ridges. In forty-eight hours a plate which contains many colonies is completely liquefied.

In Stab-Cultures the gelatine is liquefied very quickly along the line of the stab.

On Agar the growth exhibits nothing characteristic.

On Potato, at 37° C., like the vibrio of cholera, it forms a dry white patch on the surface, the growth being often only visible by reflected light.

In Bouillon it forms no pellicle.

Gelatinized Blood-Serum and *Egg Albumen* are liquefied.

Glucose is not fermented. *Indol* is not produced.

Milk containing blue litmus-tincture is almost completely decolorized in from three to four days at 37° C., with coincident coagulation of the casein and the formation of a layer of whey above it.

Pathogenesis.—Out of twenty-one animals previously treated by Koch's method with soda and tincture of opium before infection, only four died.

(*The other vibrios are to be found in the section on Water Bacteria.*)

SPIROCHÆTE OBERMEIERI.

Found by von Obermeier in the blood of patients suffering from relapsing fever.

Microscopical Appearances.—Occurs in the form of long, spiral, flexible threads, with ten to twenty "throws" in their length. The spirals measure 16 to 40 μ in length, and the germs are about $\frac{1}{3}$ to $\frac{1}{4}$ as wide as the cholera vibrio.

Motility.—Actively motile, exhibiting a twisting motion and bending occasionally at any point along their length. No flagella; the extremities taper to a fine point.

Staining Reactions.—Easily stained with fuchsin, alkaline methylene-blue, or Bismarck-brown.

Biological Characters.—Outside the body, in blood-serum and $\frac{1}{2}$ per cent. sodium chloride solution, they retain their motility for a long time. They have, however, never been cultivated on artificial media.

Pathogenesis.—Monkeys are susceptible when inoculated with human blood containing the germ, typical relapsing fever being produced. The disease can be conveyed from one monkey to another, but only with blood containing Spirochæte. Typical fever can be again produced in a monkey which receives a second injection a few days or weeks after recovery from the first injection.

BACILLUS DIPHThERiÆ.

This bacillus was first observed by Klebs (1883) in diphtheritic false membranes. It was cultivated in pure cultures, and its pathogenic properties demonstrated by Löffler in 1884.

Microscopical Appearances.—Somewhat plump rods of variable size, 1 to 6 μ long, and 0.5 to 1 μ broad, either straight or slightly curved, with rounded ends. Irregular forms are very common, and indeed are characteristic of this bacillus. In the same culture and in unfavourable media great differences in form and dimensions occur; one or both ends may appear swollen (see Fig. 77, in the centre of the field), or the central portion may be thicker than the extremities, or the rod may consist of irregular, spherical, or ovoid segments. The rods sometimes lie in clusters alongside of each other in a characteristic manner, like a bundle of faggots or a spilled box of matches. Branched forms may be observed at times, but this condition is comparatively rare.

Spore Formation absent.

Motility.—Non-motile.

Staining Reactions.—The best results are obtained with Löffler's methylene-blue, or a weak solution of carbol-fuchsin. Gentian-violet stains too intensely, obscuring the structure of the organism. The reaction with the Gram and Claudius methods is positive. Roux's double stain (see p. 51) also stains the bacilli very well. Neisser has described a double stain (see p. 24) which can be used as an aid in differential diagnosis. In sections of tissue the bacilli can be stained by Löffler's method, also by the Gram and Claudius methods.

Biological Characters.—Grows most freely in the presence of oxygen, but is also a *facultative anaërobie*. The growth takes place only at temperatures between 20° and 42° C.; the optimum temperature is about 35° C. It grows best on alkaline media. It does not liquefy gelatine or serum. Cultures may remain alive for five months.

On Gelatine Plates.—Small round white colonies develop, which under a low power appear yellowish-brown in colour, and granular, with irregular borders, rarely reaching a diameter of over 1.5 mm. The development is very slow, nothing very characteristic being observed in less than seventy-two hours.

In Gelatine Stab-Cultures.—Forms small, round, white colonies of limited size along the track of the needle.

On Agar.—It grows best on *glycerine-agar* plates. In twenty-four to forty-eight hours small, greyish-white colonies develop, which macroscopically often exhibit a stratified appearance, and under a low power appear granular, with irregular borders.

On Agar Slant Cultures.—In twenty-four hours, small, transparent, slightly elevated colonies appear, after which growth is very scanty, and does not extend far from the inoculation track.

In Agar Stab-Cultures.—Colonies develop along the track of the needle, surface growth being very limited.

In a mixture of *glycerine-agar* and *human blood-serum* the growth is much more pronounced and extensive (see Fig. 78, of a culture growing in this medium).

On Löffler's Serum (see p. 69).—In twenty-four hours somewhat large, whitish, opaque colonies of firm consistence develop, which only increase slightly in size during the next few days. This medium is the best for diphtheria bacilli, and is usually used for differential diagnosis.

In Bouillon.—The bacillus grows in fine clumps, which fall to the bottom of the tube, or are deposited on the sides without causing any clouding of the fluid. The bouillon may appear diffusely clouded to the naked eye, but when examined microscopically in a hanging-drop the clumpy arrangement is easily observed. In bouillon kept at 35° C. for some time a whitish film often forms over part of the surface. The reaction of the bouillon is subject to changes—frequently at first it is acid, and subsequently again alkaline. These changes can be observed in the bouillon when a little rosolic acid is added.

On Potato, which is rendered alkaline, a delicate coating forms.

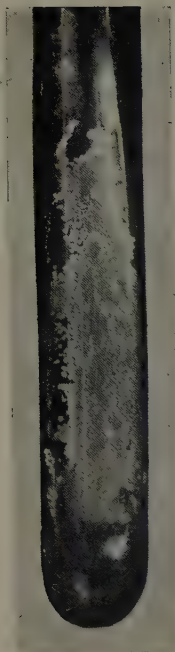


FIG. 78.—*B. diphtheriae*.
Human blood-serum-agar
culture.



FIG. 79.—*B. typhi abdominalis*. Gela-
tine stab-culture.



FIG. 80.—Avian Diphtheria. Chickens in the last stages of the disease.

Milk is a favourable medium, and is not coagulated. Schottelius reported experiments in which he added 1 c.c. of a bouillon-culture of the diphtheria bacillus to 20 c.c. of bouillon, 20 c.c. of fresh milk, and 20 c.c. of boiled milk respectively. The fresh milk was obtained direct from the cleansed cow's udder. After six hours' cultivation at ordinary temperature, 1 c.c. of each sample contained :—

Fresh milk	=	21,280.000 colonies of <i>B. diphtheriæ</i> .	
Sterilized milk	=	2,280.000	" "
Bouillon	=	7,600.000	" "
After longer incubation at 37° C. :—			
Fresh milk	=	50,160.000	" "
Sterilized milk	=	6,080.000	" "
Bouillon	=	18,240.000	" "

Diphtheria epidemics have on several occasions been traced to a milk-supply which might have been contaminated. The first case in which a milk-supply was proved to contain virulent diphtheria bacilli was reported by the author in 1897 (*Veterinary Record*). Virulent bacilli have since been found once in milk by Klein (*Journal of Hygiene* 1901, vol. i., No. 1).

In cooked and raw eggs the diphtheria bacillus develops very well both in the white and the yolk ; as also on solid egg albumen, on which it sometimes exhibits branched forms.

Vitality.—Corrosive sublimate 1 to 1000 kills cultures in thick layers within twenty seconds ; 5 per cent. solution of permanganate of potash, 5 per cent. solution of carbolic acid, and 3 per cent. carbolic acid in 30 per cent. alcohol, kills cultures in the same space of time. Lemon-juice kills the bacilli quickly. They are destroyed when heated to 60° C. for ten minutes. In thick layers they resist drying for some months. They resist ordinary cold well, but in the ice-chest they rapidly lose their power of producing toxin. According to Löffler the bacilli may remain alive in gelatine-cultures for 331 days. Abel cultivated diphtheria bacilli from a box of wooden bricks with which a child suffering from diphtheria had been playing six months before. The bacillus has also been found in soiled linen, hair, drinking glasses, etc. Diphtheritic membranes dried and kept in the dark may harbour living bacilli for months.

Pathogenesis.—Under natural conditions infection through *B. diphtheriæ* does not occur in animals ; the so-called spontaneous

chicken and pigeon diphtheria are etiologically different diseases. By inoculation into the trachea in cats and rabbits true diphtheritic lesions are produced with general toxæmia and death from absorption of the toxins formed at the seat of disease. Guinea-pigs inoculated subcutaneously with 0.1 to 0.5 c.c. of a bouillon culture die in from two to five days, showing the following appearances at autopsy:— Extensive œdema, hyperæmia, and ecchymosis at seat of inoculation; lymphatic glands congested; exudation into the pleuræ, peritoneum, and pericardium; suprarenal capsules enlarged and hæmorrhagic; spleen sometimes enlarged; fatty degeneration of liver and kidneys. Rabbits are not so susceptible, and generally recover after a small injection. When death does not follow inoculation rapidly, the visceral changes are less marked, and we often get nervous symptoms or progressive paralysis. Among common animals, rats and mice alone are immune. Though rats are not susceptible to infection, they succumb to intracerebral injection of the specific toxin, as has been shown by Borrel and Roux.

Differential Diagnosis.—Mixed infection is frequent in diphtheria, and may be due to *Streptococci*, *Staphylococci*, *Pneumococci*, and *B. coli*.

Pseudo-diphtheria bacilli, which resemble very closely in their morphology the true Löffler's bacillus, are distinguished as follows:— They are non-pathogenic for experimental animals, and when grown in alkaline bouillon do not render it acid. The genuine bacillus renders slightly alkaline bouillon acid, but the reaction may again become alkaline.

Immunity.—Fränkel was the first to immunize guinea-pigs against diphtheria by inoculating them with the specific toxin, but to Behring belongs the credit of the fundamental discovery that the blood of an immunified animal may be employed for protective inoculation, and even in larger quantity exercise a curative influence on the disease. This is one of the greatest discoveries in scientific medicine of recent years. The principle involved has since been proved to be applicable to a number of other diseases.

The diphtheria toxin used for immunizing animals which are intended to yield anti-toxic serum is obtained by cultivating the virulent diphtheria bacillus in bouillon exposed to the air. In three weeks, or longer, the culture is rich enough in toxin to be employed.

The culture is then filtered through a Chamberland filter, and the clear filtrate is preserved in well-filled vessels, which are protected from light, and kept at ordinary temperature. One-tenth c.c. of a toxin so prepared is usually fatal in forty-eight hours to a guinea-pig. It loses its activity after a time, though very slowly, if kept in the manner above mentioned. There is considerable variation in the degree of virulence of different cultures of the diphtheria bacillus. For the production of much anti-toxin the culture used should be as highly toxic as possible.

Experimental animals are easily immunized. Behring, and later Roux, immunized horses with diphtheria toxin which had its poisonous properties weakened by the addition of a solution of trichloride of iodine, or iodide of potassium. A serum possessing very high immunizing properties is obtained from the horse by introducing into that animal large quantities of diphtheria toxin as follows :—

Preparation of Anti-Toxin.

Having selected a healthy seven-year-old horse, weighing about 400 kilogrammes, the animal is treated with toxin,* the injections being made under the skin of the neck or behind the shoulders.

Days of Injection.	Injection of	Toxine with Iodide of Potash.	Reaction.
1	$\frac{1}{4}$ C.C.	I—10	No reaction.
2	$\frac{1}{2}$ "	I—10	do.
4, 6, 8	$\frac{1}{2}$ "	I—10	do.
13, 14	1 "	I—10	do.
17	$\frac{1}{4}$ "	Pure toxin.	Slight œdema, no fever.
22	1 "	do.	do.
23	2 "	do.	do.
25	3 "	do.	do.
28	5 "	do.	do.
30, 32, 36	5 "	do.	do.
39, 41	10 "	do.	do.
43, 46, 48, 50	30 "	do.	Well-marked œdema, disappearing in twenty-four hours.
53	60 "	do.	do.
57, 63, 65, 67	60 "	do.	do.
72	90 "	do.	do.
80	250 "	do.	do.

In eight to ten days after the last injection 5 to 6 litres of blood

* Roux used a toxin of which 0.1 c.c. killed a guinea-pig weighing 500 g. in forty-eight hours.

are taken from the jugular vein of the horse by means of a sterilized trocar, and placed in the ice-chest until clear serum has separated.

Behring preserves the serum thus obtained by adding 0.5 per cent. carbolic acid. Schering's serum is preserved with 0.4 per cent. trikresol, which is considered to possess about twice the antiseptic power of carbolic acid, and is only half as poisonous. In the Pasteur Institute a piece of camphor is used as an antiseptic. Serum desiccated *in vacuo* is convenient to send to a distance, being dissolved again in eight or ten times its weight of pure water before use.

Anti-toxic Treatment.

The anti-toxic power of a serum is tested by the amount of toxin of known strength which it is capable of neutralizing, and the dose is reckoned in accordance therewith. The reader is referred to special treatises for further particulars.

BACILLUS DIPHTHERIÆ COLUMBARUM.

Obtained by Löffler in 1884 from the false membranes in the mouths of pigeons. Chickens are also affected.

Symptoms.—In pigeons, reddish patches are formed on the mucous membrane of the mouth and fauces. The patches are covered later with a layer of thick yellow fibrinous exudation, the back part of the tongue, fauces, and corners of the mouth being specially affected. In chickens the tongue, gums, nares, larynx, and conjunctival mucous membranes are the parts affected (see Fig. 80, of two Plymouth Rock chickens suffering from this disease). The disease is very fatal to young fowls, the choice varieties being most susceptible.

Microscopical Appearances.—Short bacilli with rounded ends usually grouped together. They are longer and narrower than the bacillus of chicken cholera. Sections of liver show them in irregular masses in the interior of the vessels.

Motility.—Non-motile.

Spore Formation absent.

Staining Reactions.—Stain with ordinary anilin dyes, but not according to Gram.

Biological Characters.—Aërobic, non-liquefying.

On Gelatine Plates, greyish-white colonies, which under a low power resemble the typhoid bacillus.

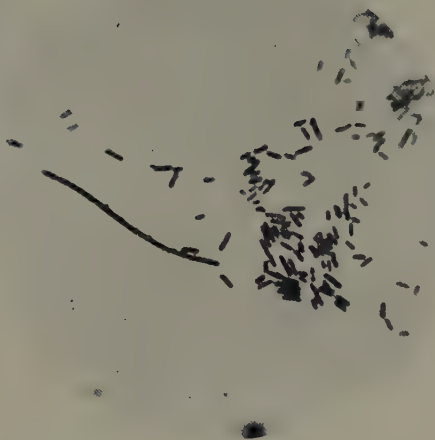


FIG. 81.—*B. typhi abdominalis*. Agar culture. $\times 1000$.

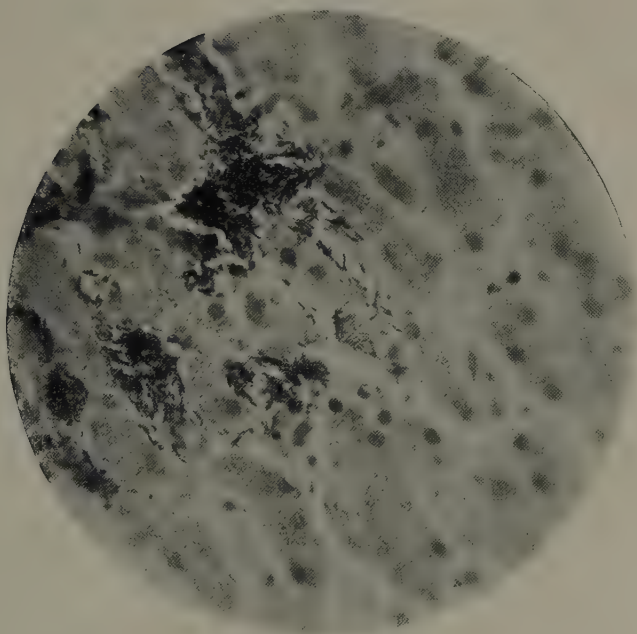


FIG. 82.—*B. typhi abdominalis*. Section of human liver. $\times 1000$.

In Gelatine Stab-Cultures, grows like a nail, with a whitish head.

On Egg, Agar, Potato, and Blood-Serum, it forms a greyish covering.

Bouillon is clouded, but there is no indol reaction.

Pathogenic for rabbits, mice, small birds, pigeons, and chickens. Rats are only slightly affected.

In experimentally inoculated mice the liver exhibits white masses of necrosed tissue containing large numbers of bacilli in the interior of the vessels. This is considered by Löffler to be of diagnostic value.

BACILLUS DIPHTHERIÆ VITULORUM.

Obtained by Löffler in 1884 from false membranes on the mucous membranes of the mouths of calves suffering from a diphtheroid disease.

Symptoms.—Yellow patches occur on the mucous membranes of the cheeks, gums, tongue, sometimes of the larynx and nares, accompanied by yellow discharge from the nose, excessive salivation, occasional coughing, and diarrhoea. The animal may die in four or five days, or may survive for several weeks. Diphtheritic patches like those in the mouth, etc., occur in the large intestine, and sometimes abscesses are found in the lungs.

Microscopical Appearances.—Bacilli five to six times as long as broad, usually in filaments..

Biological Characteristics.—This bacillus does not grow in the usual media, nor in gelatine sheep blood-serum, but when cultivated on calves' blood-serum, pieces of the affected tissue produced a whitish growth, which, however, did not grow when transferred afresh to serum.

Pathogenesis.—Fatal for mice in from seven to thirty days when inoculated subcutaneously. The autopsy reveals extensive infiltration of abdominal walls, which often spreads into the peritoneal cavity, enveloping the viscera in a yellowish exudation. The bacilli are found in this exudation, and mice inoculated with some of the fluid die with similar lesions. Non-pathogenic for rabbits and guinea-pigs.

BACILLUS TYPHI ABDOMINALIS.

This organism was first observed by Eberth in the internal organs

of persons dead of typhoid fever. Koch also saw the bacillus about the same time, and photographed it. It was first obtained in pure cultures by Gaffky, and has also been found during life in the fæces, urine, rose spots, and occasionally in the blood of typhoid patients, as also in post-typhoid abscesses.

Microscopical Appearances.—Short, plump rods with rounded ends, 1 to 3 μ long, and 0.5 to 0.9 μ broad, which in sections of tissue are usually found singly, but in cultures often occur in long threads. (See Fig. 81.) In agar cultures at 37° C., in the bodies of animals, and in human tissues, the rods are more plump, and smaller in all directions than on gelatine and potato media, long threads being encountered more frequently in cultures maintained at low temperatures.

Motility.—Actively motile, each bacillus possessing eight to eighteen flagella, given off from over the whole surface of the organism. (See Figs. 83 and 84, stained by the writer's orcëin method.)

Spore Formation unknown. The so-called spores of Gaffky are now considered to represent involution forms.

Staining Reactions.—With the ordinary anilin dyes the rods do not stain so readily as most other organisms. Watery solutions of the dyes and a weak solution of carbol-fuchsin give the best results when slightly heated during the staining process. The bacilli do not stain by the Gram method. Small vacuoles are sometimes present in the rods, due to retraction of the protoplasm from the cell envelope. The bacilli are easily detected in the tissues, especially in pieces of the liver or spleen, where they can be observed, massed together in characteristic clumps, when stained with carbol-fuchsin. The staining is done at room-temperature, or at 40° to 45° C., after which the specimens are washed in absolute alcohol, cleared in xylol, and mounted in xylol-balsam. Alkaline methylene-blue can also be used. The characteristic clumps in the tissues consist of small oval or rod-shaped organisms closely packed together, individual bacilli being often only visible at the periphery of the mass, usually in the neighbourhood of a capillary.

Biological Characters.—The typhoid bacillus grows on ordinary nutrient media at any temperature between 20° and 38° C. The growth is most luxuriant at 37°, while the development is very slow at

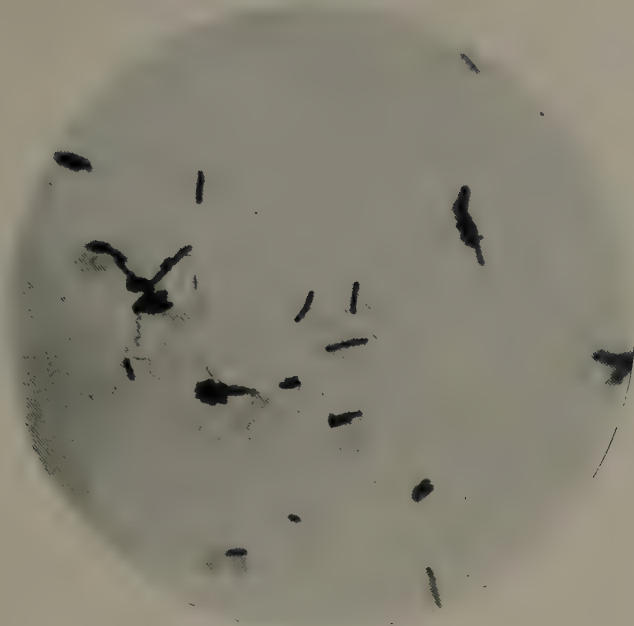


FIG. 83.—*D. typhi abdominalis*, showing flagella.

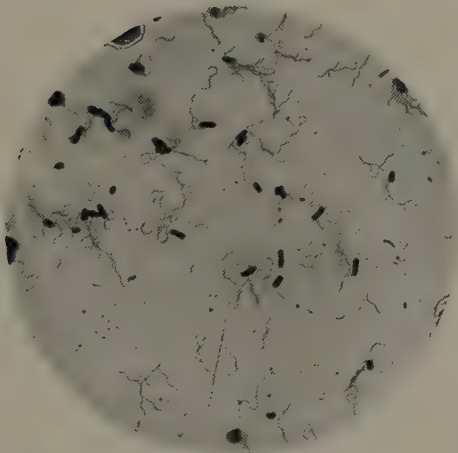


FIG. 84.—*B. typhi abdominalis*, showing flagella.

room-temperature. The bacillus is a facultative anaërobe, and grows fairly well in an atmosphere of CO_2 . In contrast to most other pathogenic bacteria, it grows luxuriantly on slightly acid media. It does not liquefy gelatine.

On Gelatine Plates the deep colonies are small, punctiform, and sharply circumscribed; under a low power they exhibit a brownish colour. The superficial colonies are much larger, forming a bluish-white, iridescent, fine coating with irregular borders, denser in the centre than at the periphery, and under a low power exhibit a brownish colour and wrinkled appearance.

In Gelatine Stab-Cultures the growth is mostly limited to the surface, with limited, thready, granular outgrowths along the track of the needle. It is often of a yellow or yellowish-brown colour (see Fig. 79).

On Gelatine Stroke-Cultures a fine, iridescent, bluish growth extends from the centre, and soon covers the whole surface of the gelatine.

On Agar and Blood-Serum a thick coating develops, which presents no typical characteristics.

On Potato.—The growth upon the surface of a cut potato is almost invisible. If examined closely, it will be found that the whole surface of the potato is covered with tufts, which, when examined microscopically, will be found to consist of numerous motile rods. This peculiarity of growth occurs, as far as is known at present, exclusively in this bacillus. Sometimes, though rarely, the growth is visible, for there are some kinds of potatoes upon which the bacilli develop a raised circumscribed tuft of a yellowish or brownish colour. These potatoes possess either a neutral or alkaline reaction, while the typical growth is confined to those exhibiting an acid reaction.

In Milk the reaction becomes acid, but there is no coagulation. *Bacillus coli*, on the other hand, causes an acid reaction and coagulation within twenty-four to forty-eight hours at 37°C .

Bouillon is clouded, slightly acidified, and a quantity of sediment forms in the tube.

In Grape-, Milk-, and Cane-Sugar Media no fermentation takes place. According to Hellstrom, 4 per cent. milk-sugar added to 1 to 4 per cent. peptone media, and inoculated with the typhoid bacillus, yields much smaller colonies than *B. coli* under similar conditions (twenty-four hours at 37°C).

On *Holz's Potato-Gelatine* (see p. 61) the growth of both the typhoid and colon bacillus is more pronounced than on ordinary bacteria. Elsner added 1 per cent. potassium iodide to Holz's medium, and found that the colon bacillus then grew more energetically than the typhoid bacillus, forming dark brown colonies in forty-eight hours, whereas the colonies of the typhoid bacillus appear as clear, watery drops. This growth is, however, not absolutely constant, further identification with other culture-methods being necessary.

Vitality.—In sterilized water the typhoid bacilli live as long as three months, and increase in numbers at first. In ordinary water they are destroyed in about fourteen days by the concurrence of the ordinary water bacteria, death taking place more quickly in running water. Under favourable circumstances, protected from light and drying, they live a long time. In fæces they appear to live three months or more, depending upon the number of putrefactive organisms present. They can withstand cold very well, though they are destroyed by repeated freezing and thawing. They are not so resistant to heat, being destroyed with certainty in ten minutes at 60° C., and in a shorter time at higher temperatures.

Specific Reactions.—1. Indol is only produced when the typhoid bacillus is cultivated according to Peckham's method of repeated transplantation at short intervals in Dunham's peptone solution. Chantemesse has also observed indol formation in old cultures.

2. Produces no gas in grape-sugar bouillon in the fermentation tube at 37° C.

3. On lactose-litmus-agar, pale blue colonies develop with no reddening of the surrounding medium; but if glucose is used instead of lactose, both the colonies and the surrounding medium become red.

4. *Gruber and Widal Reaction.*—When typhoid immune serum is added to a bouillon culture of the typhoid bacillus in the proportion of one to forty, the organisms become agglutinated, the bacteria forming granular masses at the bottom of the test-tube, while the upper portions of the medium remain clear. Agglutination is accompanied by loss of motility. This constitutes Gruber's reaction. Widal has applied this reaction to diagnostic purposes, it having been found that agglutination is also produced by even at times highly diluted serum (1.50, 1.500, to 1.2500) of typhoid patients. (See Figs. 85 and 86.)

When making an agglutination test it is necessary to obtain a

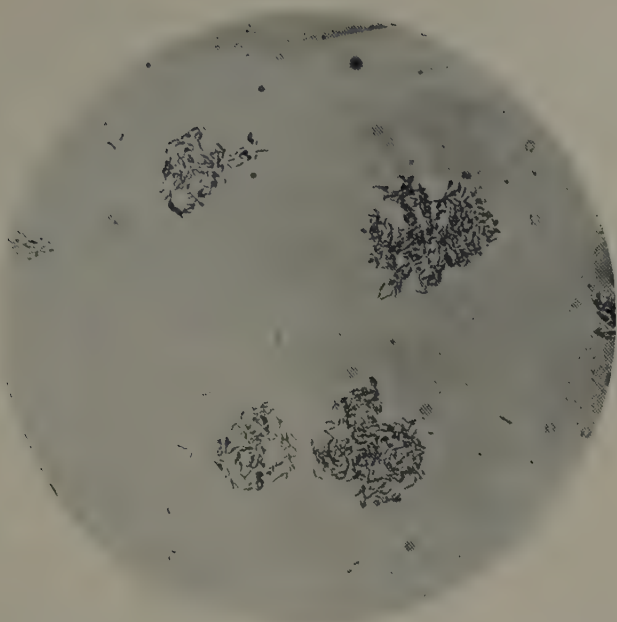


FIG. 85.—*B. typhi abdominalis*. Agglutination reaction. Stained with aqueous solution of gentian violet. $\times 400$.



FIG. 86.—*B. typhi abdominalis*. Agglutination reaction. Stained with aqueous solution of gentian violet. $\times 1000$.

homogeneous suspension of the typhoid bacillus. This suspension is prepared by taking a small platinum loop full of a culture not more than twenty-four hours old, grown on rather dry agar, and carefully rubbing it up in a drop of the bouillon against the side of a tube containing 1 c.c. of bouillon, and subsequently mixing the drop with the rest of the bouillon. A control preparation is examined microscopically to make sure there are not clumps in the emulsion. A small drop of the suspension is then placed on a cover-glass, and a drop, as near the same size as possible, of diluted serum of a typhoid patient is mixed with it, a hanging-drop being prepared from the mixture in the usual manner. Microscopically examined, the bacilli will be observed to gradually form groups of three or four, which, through the addition of other bacilli, constantly increase in size, until the majority are in "clumps." If a marked reaction occurs within thirty minutes, the case is one of enteric fever, but without great experience it is impossible to say that the absence of this reaction negatives such a diagnosis. When the results are negative more than one examination should be made, for it occasionally, although rarely, occurs that the reaction is seen on one day and not on another.

Wyatt Johnston has modified this method, and uses a watery solution of dried blood-serum from typhoid patients. The crust of blood is covered with a drop of water, and on standing for one or two minutes a drop of this is mixed with one loopful of a typhoid culture, a second loopful being added later. The agglutination test is also frequently used for the determination of the typhoid bacillus when other tests give doubtful results.

The Examination of Water for Typhoid Bacilli is essential in all outbreaks of typhoid fever, for water has been shown to serve as a vehicle for the typhoid bacillus. For this examination, carbolic acid is added to the nutrient medium (gelatine or agar) in the proportion of 0.05 to 0.25 per cent., as this addition serves to inhibit the development of the ordinary liquefying water bacteria, while the typhoid bacillus and some allied forms grow in presence of a small amount of carbolic acid. Plate cultures are then made in the usual way. Elsner's method serves the same purpose.

To get positive results large quantities of water may have to be examined, and this is best done as follows: A concentrated sterilized alkaline peptone and salt solution is prepared, so that when added to 100 c.c. of the water the latter has the composition of Koch's peptone-

water. The water, which is contained in sterilized Erlenmeyer flasks, is then placed in the incubator for eighteen to twenty-four hours. Under these conditions the growth of any typhoid bacilli is allowed to go on practically without competition, and there is a greater likelihood of isolating the typhoid bacillus in plate cultures.

Pathogenesis.—When a virulent culture is inoculated into mice, guinea-pigs, rabbits, and goats, it produces spasms, a fall of temperature, diarrhœa, and death if the dose is sufficiently large. By subcutaneous injection large quantities of the culture are necessary, but a small quantity is sufficient when intraperitoneal and intravenous inoculations are made. In most cases in animals death occurs without the appearance of typical pathological changes, the fatal result in most cases being due to a toxic action of the virus. Sanarelli found that rabbits, guinea-pigs, and mice were rendered susceptible to infection when first inoculated with the products of the growth of certain saprophytes (*Proteus vulgaris*, *Bacillus prodigiosus*, and *Bacillus coli*), and afterwards with fresh cultures of the typhoid bacillus. The inoculation was followed by death in twelve to forty-eight hours, with well-marked pathological changes in the digestive tract, especially in the small intestines. The infection is general in such cases, and the bacilli can be recovered from the blood and internal organs.

BACILLUS COLI COMMUNIS.

This bacillus is a normal inhabitant of the intestines of man, cattle, swine, dogs, and a variety of other animals. It is also found associated with diseased conditions, such as inflammatory and suppurative processes in the peritoneal cavity, infectious enteritis, affections of the liver, puerperal fever, broncho-pneumonia, empyema, endocarditis, meningitis, cystitis, and pyelo-nephritis.

Microscopical Appearances.—Occurs in short motile rods, mostly in pairs (see Fig. 89), sometimes in threads.

Motility.—The rods possess numerous long flagella (see Fig. 87).

Spore Formation is absent.

Staining Reactions.—Stains with the ordinary anilin dyes, but not by either the Gram or Claudius method.

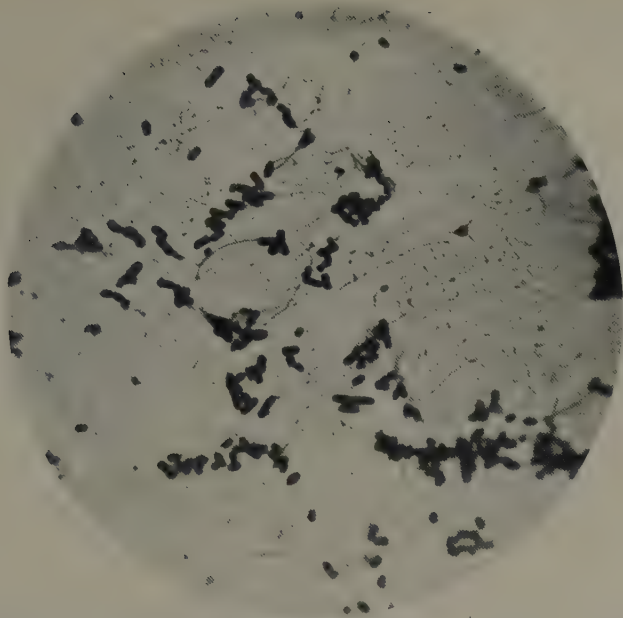


FIG. 87.—*B. coli communis*, with flagella. Agar culture. Bowhill's stain. $\times 1000$.

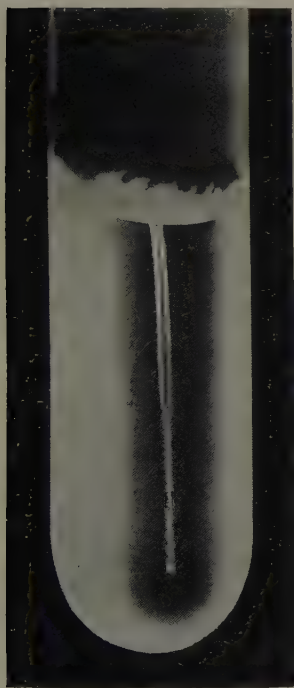


FIG. 88.—*B. coli communis*. Gelatine stab-culture.

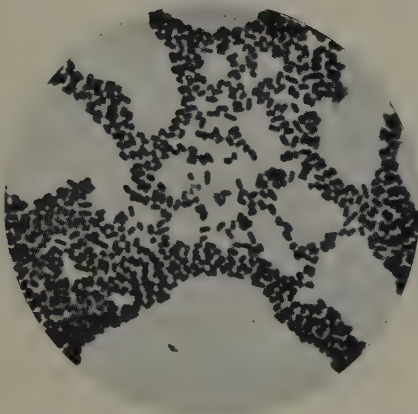


FIG. 89.—*B. coli communis*. Agar culture. Fuchsin. $\times 1200$.

Cultivation.—In cultures the organism grows under both aërobic and anaërobic conditions, and when grown anaërobically forms gas in the media.

On Gelatine Plates the surface colonies are iridescent with wavy borders; deeper colonies are round and of a brownish colour; the gelatine is not liquefied.

In Gelatine Stab-Cultures it grows in the form of a nail with limited growth along the track of the needle (see Culture, Fig. 88).

On Agar it forms a grey coating.

On Potato the growth is of a brownish colour.

In Bouillon it causes diffuse clouding.

In Milk it produces acid and causes coagulation, which generally takes place in about thirty-six hours.

Special Reactions.—Indol is formed in bouillon, as also in Dunham's peptone solution, in forty-eight to seventy-two hours. The bacillus causes fermentation in milk-sugar, grape-sugar, and glycerine media.

On Lactose-Litmus Agar the colour of the colonies is pink, and that of the surrounding medium blue-red.

Pathogenesis.—Affects mice, guinea-pigs, and rabbits, the pathogenicity of the bacillus varying.

For the differentiation of the *Bacillus coli* and *Bacillus typhi* Piorkowski prepares cultures in bouillon, gelatine, and agar, prepared with urine, to which the desired quantity of peptone, gelatine, and agar is added. Cesaris-Demel (*Giornale della R. Accad. di Medicina di Torino*, 1898, No. 3) describes a method for the differential diagnosis of the typhoid bacillus and *B. coli* as follows: Cultures of the organisms are prepared in bouillon made from calf's liver. The sugar present in the liver will be fermented by *B. coli*, and it will exhibit a rapid luxurious growth, while on this medium the growth of the typhoid bacillus is confined to very narrow limits.

DIFFERENTIAL DIAGNOSIS TABLE.

	BACILLUS TYPHI ABDOMINALIS.	BACILLUS COLI COMMUNIS.
(1) Motility	Usually very pronounced; large numbers of flagella, staining without much difficulty with orcein	As a rule not very pronounced, sometimes absent; flagella demonstrated with difficulty, fewer and shorter
(2) Cultures	On gelatine plates the typhoid colonies develop somewhat slowly, while on potatoes they are as a rule invisible (not always) Does not coagulate milk; slight acid reaction; may become alkaline later	On gelatine the colon bacillus develops more rapidly than the typhoid, and on potatoes it grows luxuriantly and is always visible Coagulates milk; marked acid reaction in thirty-six to forty-eight hours in the incubator
(3) Fermentation	Causes little or no gas-formation in media containing glucose, lactose, or saccharose	Forms much gas in glucose, lactose, and saccharose media
(4) Changes observed in agar or gelatine medium containing lactose and litmus tincture of slightly alkaline reaction	The colonies are of a pale blue colour, and there is no reddening of the surrounding medium	The colonies are pink, and the surrounding medium red
(5) Indol	Does not <i>as a rule</i> produce indol in peptone solutions	Produces indol in solutions in forty-eight to seventy-two hours at 37° to 38° C.
(6) Agglutination	Positive reaction with the serum of a typhoid patient or of an animal immunized against B. typhi	Reaction negative with few exceptions
(7) Pfeiffer's reaction with typhoid serum	<i>Positive</i>	<i>Negative</i>

BACILLUS EQUI INTESTINALIS.

Found by Dyas and Keith in the intestines of a horse. It is distinguished from the *B. coli* as follows:—It is somewhat thicker, does not grow at a low temperature, and produces no gas in fermentation tubes. It coagulates milk in one to two days.

BACILLUS INFLUENZÆ.

Pfeiffer discovered this bacillus and isolated it in pure culture during the epidemic in 1891-92.

Microscopical Appearances.—Very small bacilli 0.3 μ wide by 1.5 μ long, with rounded ends. It very seldom forms threads in sputum, but does so frequently in fresh cultures; involution forms are already visible in three to four-day old cultures.

Motility.—Non-motile.

Spore Formation unknown.

Staining Reactions.—The bacillus stains with difficulty. Löffler's methylene-blue is a good stain, but dilute watery solution of carbol-fuchsin is better. The preparation must be stained for five to ten minutes; if stained for a shorter time, or treated with other stains, the ends of the bacillus only take up the colour perfectly. The results with the Gram method are negative.

Biological Characters.—The influenza bacillus is aërobic, and grows only in the presence of blood or pus, which explains why the cultivation of the influenza bacillus was so long a failure. Pfeiffer was able sometimes to obtain cultures from lung pus direct on agar, but at other times it was impossible. The cause of the irregularity was that the rods in the first cultures developed when a trace of blood was inoculated with the material, there being no growth when no blood was present. The influenza bacillus can be cultivated regularly and transferred through several generations in nutrient media containing blood. Blood-agar is the best medium. To obtain pure cultures Pfeiffer employed the following method :—

The bronchial secretion or exudate from the broncho-pneumonic infiltrated portion of the lung in influenza-pneumonia is thoroughly emulsified with 1 to 2 c.c. of bouillon. Several platinum loops of the bouillon are inoculated, and thoroughly spread over the whole surface of blood-agar media, and at the same time control cultures are prepared on ordinary glycerine-agar medium. The dilution of the bouillon has the effect of segregating influenza bacilli so that they grow in separate colonies on the blood-agar medium, while any hæmoglobin present in the original material is so thoroughly diluted, that the influenza bacillus cannot develop on the control agar tubes containing no blood.

The inoculated tubes are placed in the incubator, and in twenty-four hours colonies appear as transparent drops on the surface of the medium. The control tubes are either sterile or contain colonies of streptococci, diplococci, or other bacteria associated with the influenza bacillus in the original material. The drop-like colonies are mostly so small that they can only be distinctly seen with a hand lens. They possess a slight tendency to become confluent, and when closely aggregated they coalesce into large drops. When the colonies are widely separated from each other, they sometimes develop until they are as large as a pin-head, but they nevertheless retain their glassy

transparent appearance. The condensation water in the tubes remains usually clear, except when it is mixed with blood that has flowed down the oblique surface of the medium, then delicate white flakes develop in it.

In Bouillon mixed with blood and spread out in a thin layer the growth is somewhat abundant.

Plate Cultures are useful for the isolation of the influenza bacillus for diagnostic purposes. A little blood is added to the liquefied agar before it is inoculated, or if Petri-dishes are used the agar is allowed to set and some blood spread on the surface, and several stroke cultures made with the diluted sputum. The developing colonies have the same appearance as those in the agar tubes.

The optimum temperature for the growth of the influenza bacillus is 37° to 38° C., the maximum about 42° C., whilst the minimum temperature is 26° to 27° C. At room-temperature no growth takes place. Oxygen is always necessary for the growth of the influenza bacillus, while no growth occurs in presence of hydrogen and CO_2 with the addition of blood to the media. Pfeiffer found that when he used blood-serum or blood-fibrin instead of blood the results were negative, and in further experiments he found that hæmoglobin was the necessary factor in the development of the influenza bacilli, as hæmoglobin agar was just as good a medium as blood-agar. Pfeiffer also obtained positive results with the blood of rabbits, guinea-pigs, pigeons, and fish, the growth with the pigeon blood being more luxuriant and quicker than with human blood, owing to pigeon's blood being very rich in hæmoglobin.

Vitality.—Heated to 60° C. the influenza bacilli die in a few minutes. In non-sterile drinking water the bacilli die in from twenty-four to thirty-six hours. On blood-agar and in bouillon they live for fourteen to eighteen days. In blood or sputum dried at 37° they are killed in one to two hours, and at room-temperature in thirty-six to forty hours.

Pathogenesis.—Pfeiffer experimented with mice, rats, guinea-pigs, rabbits, swine, cats, dogs, and monkeys; and only in monkeys was he able to produce a disease simulating influenza, by inoculating them through the chest wall direct into the lungs, and also by what is a more natural mode of infection, in one monkey, by the introduction of the influenza culture into the nose. The animal had fever and slight

coughing for several days, but the inoculated bacilli did not multiply. Large doses kill rabbits through intoxication, the temperature falling rapidly before death.

Immunity.—In Pfeiffer's experiments monkeys did not react so strongly to a second injection of influenza bacilli as they did to the first; this fact he considered an indication of acquired immunity. Man can with certainty be attacked several times with influenza; sometimes the same individual may have several attacks during the course of one epidemic. It follows, then, that in man there is at any rate no lasting immunity after an attack of influenza.

PSEUDO-INFLUENZA BACILLUS.

Found by Pfeiffer in a case of broncho-pneumonia in a diphtheritic child. The bacillus resembled the influenza bacillus in its growth on blood-agar, its appearance and staining reaction. Similar bacilli have also been isolated by other investigators in *otitis media* and influenza. Pfeiffer considers that they are allied to the influenza bacillus, and designates them pseudo-influenza bacilli. They are distinguished from the genuine influenza bacilli by their growth on culture media being much more pronounced after twenty-four hours, and by their tendency to form long filaments, a condition rarely occurring in cultures of the genuine bacilli.

The Septicæmia Hæmorrhagica Group of Bacteria.

BACILLUS BOVISEPTICUS.

(*Ger.* Wildseuche, Rinderseuche.)

This disease occurs in two forms: one form is characterised by œdema of the skin and subcutaneous tissue—particularly of the head—and swelling of the tongue; the second or pectoral form is characterised by pleuro-pneumonia, swelling of the interstitial tissue of the lungs, pleuritis, and pericarditis. Both forms generally run into hæmorrhagic enteritis. The mortality is about 90 per cent. The bacillus was first observed by Kitt, and further studied by Kitt and Hueppe, and classified as one of the septicæmia hæmorrhagica group of organisms. The Italian buffalo disease (*Barbone dei bufali*) is probably due to the same organism. The organism is similar to that which produces "*Schweine-seuche*," and in fact has been regarded as identical by some authorities,

for the reason that in several instances both organisms proved pathogenic for calves and pigs.

BACILLUS OF SEPTIC PLEURO-PNEUMONIA OF CALVES.*

This disease is rapidly fatal in very young calves. The lesions produced are somewhat analogous to those of pleuro-pneumonia contagiosa, but the thickening of the interlobular connective tissue is less marked, and the exudation of lymph not so abundant. There is also a want of uniformity of colour in the individual pulmonary lobules in some cases. Sero-fibrinous exudates and pleuritic adhesions have been frequently observed, and it is also stated that inflammation of the pericardium, liver, kidneys, stomach, and intestines occasionally occur.

Microscopical Appearances.—In the lung and muco-pus of the bronchi small ovoid organisms are present, with rounded ends, $1\ \mu$ to $1.5\ \mu$ long, and $0.5\ \mu$ thick.

Staining Reactions.—Stain easily with the ordinary anilin stains, but not by Gram's method. When weak solutions of gentian-violet are used, the organisms exhibit bipolar staining which is characteristic of the septicæmia hæmorrhagica group of bacteria.

Motility.—Very motile.

Biological Characters.—Grows well in bouillon and on solid media.

Pathogenesis.—Rabbits infected by ingestion or inoculation die in twenty-four to forty-eight hours. Intrapulmonary injection of one drop produces pneumonia. Calves also died by intrapulmonary injection; sheep and dogs are immune. It is a facultative parasite capable of living in the soil, which, according to Poels, explains the presence of the disease on an infected farm.

BACILLUS DYSENTERIÆ VITULORUM.†

(Bacillus of White Diarrhœa of Calves—Jensen.)

Described by Jensen as the cause of the so-called white diarrhœa or scour occurring amongst calves.

Microscopical Appearances.—Small bacilli a little larger than the chicken-cholera bacillus.

* See Poels, *Fortschr. d. Med.*, 1886, iv., 388; Jensen, *Monatsh. f. Thierheilk.*, 1890; Liénaux, *Ann. de Méd. Vétér.*, 1892, p. 465; etc.

† See Jensen, *Monatsh. f. Thierheilk.*, 1893, iv., p. 97.

Motility.—Non-motile.

Staining Reactions.—Polar staining with the ordinary reagents ; Gram method negative.

Biological Characters.—The growth is luxuriant on the usual media, and very similar to that of *B. coli*.

On Potatoes it forms a brown-coloured shiny growth ; the cultures give off gas possessing an unpleasant odour.

Pathogenesis.—When new-born or very young calves are fed with 5 c.c. of a bouillon culture, a deadly diarrhœa is produced, death occurring in the course of one to two days, the bacilli being found in the intestines and organs. In sections they were observed in clusters in the small bloodvessels.

BACILLUS CHOLERÆ COLUMBARUM.*

This organism was found by Leclainche in an epidemic among wild pigeons, and is probably a variety of the fowl-cholera bacillus.

Microscopical Appearances.—Similar to those of the fowl-cholera bacillus, but a little larger.

Motility.—Non-motile.

Spore Formation absent.

Cultivation.—*On Gelatine* and *Agar* growth is similar to fowl cholera bacillus.

Bouillon is not clouded, but a flaky sediment is formed.

On Potatoes at 20° C. it forms a greyish-yellow layer.

Pathogenesis.—Wild pigeons are most susceptible, dying of septicæmia in three to six days after infection through feeding ; and in two days when injected intravenously. The symptoms are drowsiness, diarrhœa, and convulsions. The tame pigeon is not so liable to infection. Fowls are immune, also dogs and cats. Rabbits, when subcutaneously inoculated, die in about eight days, and guinea-pigs in about ten days.

BACILLUS OF DUCK CHOLERA.

This organism was found by Cornil and Toupet in an epidemic among the ducks in the Jardin d'Acclimatation at Paris. The disease

* Leclainche, *Ann. de l'Inst. Pasteur*, 1894, p. 490.

was characterised by diarrhœa, feebleness, and muscular tremors, and terminated fatally in two to three days.

Microscopical Appearances.—Morphologically, and in its behaviour to stains, it resembles the bacillus of fowl cholera. Non-motile, and does not form spores.

Cultivation.—The growth in the various media corresponds to that of the bacillus of fowl cholera. (See below.)

Pathogenesis.—Affects ducks, but not chickens or pigeons, and only kills rabbits when injected in large quantities. Ducks die in one to three days from subcutaneous inoculations, or by ingestion of food containing the bacillus.

BACILLUS CHOLERÆ GALLINARUM.*

(Fowl Cholera ; *Ger.* Hühnercholera ; *Fr.* Cholera des poules.)

Chicken or fowl cholera is a disease which often occurs in poultry as an epizootic ; it is characterised by diarrhœa, and death in one to two days.

Microscopical Appearances.—Very short non-motile bacilli of varying size, which do not form spores.

Staining Reactions.—In cover-glass specimens the bacilli stain with the ordinary anilin dyes, more at the poles than in the middle, giving them the appearance of diplococci (see Fig. 90), but by intensive staining they appear as genuine bacilli. They do not stain by the Gram method.

Vitality.—Exhibit slight resistance to heating and drying, but remain alive a long time in cultures.

Biological Characters.—An aërobic, non-liquefying bacillus, growing in the usual culture media at both room-temperature and in the incubator. Indol is produced in cultures.

On Gelatine Plates, in the deep portions of the medium, they form round, irregular, brownish discs. Growth on the surface is slow and limited.

In Gelatine Stab-Cultures the growth occurs on the surface, as well as along the course of the needle. The surface growth consists of a delicate greyish-white coating.

* See Pasteur, *Compt. Rendus*, 1880, xc., p. 239, 952, 1030 ; xci., p. 673 ; Gamaleia, *Centrabl. f. Bakteriöl.*, 1888, iv., 161 ; etc.

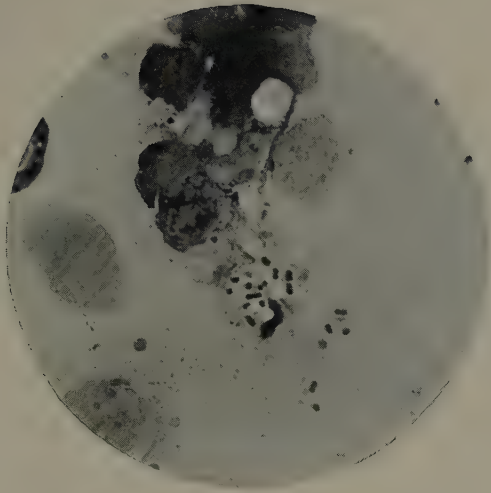


FIG. 90.—Bacillus of Fowl Cholera. Cover-glass specimen from inoculated mouse. Fuchsin. $\times 1000$.

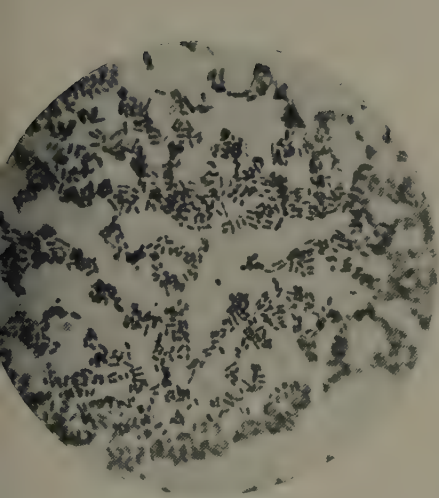


FIG. 91.—*B. phasianii septicus*. Cover-glass specimen. Agar culture. Fuchsin. $\times 1000$.

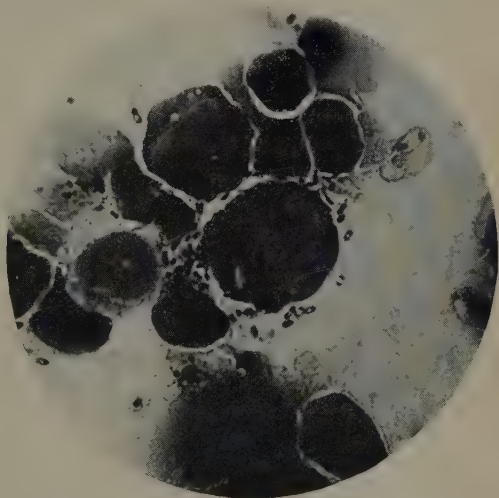


FIG. 92.—Bacillus of Swine Fever. Spleen of pig. Methylene blue. $\times 1000$.

On Agar and Blood-Serum a glistening whitish coating is formed.

On Potatoes it does not grow at room-temperature, but in the incubator a transparent, greyish-white, flat coating is formed.

Bouillon is slightly clouded.

Milk is gradually coagulated and acid formed.

Pathogenesis.—The bacilli cause typical septicæmia by cutaneous inoculation of minute quantities of culture, and in larger doses by feeding. Pigeons, fowls, geese, ducks, pheasants, small birds, also birds of prey, rabbits, and mice are susceptible. Guinea-pigs, sheep, and horses exhibit only a local reaction, suppuration occurring at the point of inoculation. Dogs and cats can devour large quantities of the cadavers of infected animals without becoming sick. Men can also tolerate the infected meat.

The bacilli are present in enormous quantities in the bloodvessels of affected animals. In pigeons, and especially in chickens, the point of inoculation is greatly inflamed, tending to necrosis. In the intestines a hæmorrhagic enteritis occurs. Chickens and rabbits exhibit pneumonic lesions. Pericarditis and hæmorrhages on the pericardium are common. The spleen and liver are enlarged. The bacilli pass from the mother to the fœtus.

Immunity.—Pasteur found that cultures of fowl cholera bacilli become attenuated if left exposed to the air for a long time. Birds inoculated with attenuated cultures are only locally affected, and become immune against infection with virulent cultures.

BACILLUS PHASIANI SEPTICUS.*

This organism was discovered by E. Klein in an epizootic amongst young pheasants. The author isolated a morphologically identical organism in an outbreak amongst some young pheasants, the mortality being very great. The principal lesions were catarrhal inflammation of the bowels and enlargement of the liver, with slight broncho-pneumonia.

Microscopical Appearances.—Small bacilli, very like the *Bacillus coli*, but smaller and shorter (see Fig. 91). The bacilli are actively motile, and do not form spores.

Staining Reactions.—Easily stained with the ordinary anilin dyes, but not by the Gram method.

* See Klein, *Journ. of Pathol. and Bacteriol.*, 1893, ii., p. 214.

Biological Characters.—Similar to those of *Bacillus coli*, except that milk is not coagulated.

Pathogenesis.—In the natural course of the disease, death takes place among young pheasants in a few days or within a week. Young pheasants are killed in twenty-four hours when inoculated with a few drops of a bouillon culture. The animals show symptoms of drowsiness and stupor. Diarrhœa is an inconstant symptom; septicæmia also occurs. Young chickens, pigeons, rabbits, and guinea-pigs do not die when injected with $\frac{1}{2}$ c.c. of bouillon culture.

It is distinguished from the bacillus of chicken cholera by its motility, its slight pathogenic action on the most of animals, and the fact that it does not coagulate milk.

BACILLUS OF GROUSE DISEASE.*

This is an infectious disease affecting *red* grouse, and is due to a bacillus discovered by E. Klein. The affected animals exhibit pneumonic lesions; the mucosa and serosa of the intestines are congested; the liver is congested and dark coloured. The bacilli are present in the bloodvessels and extravasated blood.

Microscopical Appearances.—Bacilli 0.4 by 0.6 by 1.6 μ , oval or coccus-like; sometimes a few are rod-shaped.

Motility.—Motile in recent cultures. When some days old, only a few of the bacilli are motile.

Spore Formation absent.

Staining Reactions.—Stains with the ordinary stains, but not by the Gram method.

Biological Characters.—On *Gelatine Plates* the surface colonies are irregular, and the deep colonies are small and round.

In Stab-Cultures.—Nail-like culture with flat head.

On Agar.—A thin greyish coating.

Bouillon becomes clouded.

In Grape-Sugar Media gas is formed.

Pathogenesis.—Very virulent for mice, and not so virulent for guinea-pigs, by subcutaneous injection. The bacilli soon lose their virulence, but soon regain it, when cultivated in bouillon to which some small pieces of hard-boiled egg-albumen is added. The yellow-hammer

* See Klein, *Centrabl. f. Bakteriöl.*, 1889, vi., 36, 593; 1890, vii., p. 81.

and finch are easily affected by subcutaneous injection, while sparrows are not so susceptible. Feeding experiments yield no positive results. Infection occurs probably by inhalation.

BACILLUS OF CANARY-BIRD SEPTICÆMIA.*

Microscopical Appearances.—Bacilli somewhat larger than the bacillus of chicken cholera, 1.2 to 2.5 μ long.

Motility.—Motile.

Staining Reactions.—Stains with the ordinary stains, most intensely at the poles, but not by the Gram method.

Biological Characters.—The growth on the various media is more luxuriant than that of the bacillus of chicken cholera.

On *Potatoes* it forms a yellow-grey coating.

Vitality.—The cultures are killed when heated for five minutes at 100° C.

Pathogenesis.—Mice die of septicæmia when fed or inoculated cutaneously or subcutaneously with minute quantities of culture. Affected canaries exhibit a fuliginous colouring of the skin and multiple necroses of the liver. Bacilli are present in great quantities in the blood.

Differential Diagnosis.—It is distinguished from the bacillus of chicken cholera by the motility of the organism and its growth on potatoes.

BACILLUS OF PNEUMO-PERICARDITIS OF THE TURKEY.†

This organism was described by MacFadyean in an epizootic among turkeys characterised by the presence of pneumo-pericardial lesions.

Microscopical Appearances.—In the blood, tissues, and artificial culture media, it occurs as short, motile, ovoid bacilli, not distinguishable by shape or size from the bacilli of fowl cholera. The bacillus does not form spores.

Staining Reactions.—Exhibits bipolar staining with the ordinary anilin dyes, but is decolorized by the Gram and Weigert methods.

Cultivation.—*Stab-Cultures in Gelatine* kept at 25° C. exhibit a

* See Rieck, *Deutsche Zeitschr. f. Thiermed.*, 1889, xv., p. 60.

† See MacFadyean, *Journ. of Compar. Pathol. and Therap.*, 1893, vi., 334.

distinctly visible growth in forty-eight hours along the track of the needle. The gelatine is not liquefied.

In Streak-Cultures in Gelatine it develops in a whitish line, which does not spread far from the track of the needle.

On slanted Agar at 37° C. it develops a thin translucent pellicle.

Bouillon becomes turbid in twenty-four hours at 37° C., a ropy sediment finally forming, and the upper portions of the bouillon becoming clear.

On Potatoes no appreciable growth takes place.

The bacillus is a facultative anaërobe, growing abundantly in bouillon in the presence of an atmosphere of hydrogen.

Pathogenesis.—The results of inoculation in guinea-pigs and rabbits are similar to those obtained with the bacillus of fowl cholera, the bacillus being very virulent for rabbits, but less so for guinea-pigs. Infection is not produced by feeding. Fowls and pigeons are slightly affected. Other birds are immune. A calf and a pony inoculated by MacFadyean manifested only slight lesions at the point of inoculation. In turkeys inoculated with pure cultures, there are symptoms of stiffness, weakness, nasal catarrh, rattling in the throat, followed by the appearance of milk-white evacuations. At autopsy, pneumonia and pericarditis are observed. Great numbers of the bacilli are present in the lungs and other organs. The bacillus is different from that of fowl cholera by being motile, by its localisation in the lungs, and slight action in other birds besides turkeys.

PNEUMO-ENTERITIS OF SHEEP.*

According to Galtier, this disease in sheep is caused by the same organism as swine fever or hog cholera. It sometimes occurs as an epizootic, causing great ravages in affected flocks. Outbreaks have been observed when recently purchased swine that had contracted swine fever in the market pens have been placed in sheep-folds. When once established, the disease transmits itself with great rapidity from sheep to sheep, and it is more severe and more frequently fatal in young animals. The general symptoms are lassitude, general loss of vigour, high fever, and absence of rumination. These symptoms are soon followed by tympany, fœtid and exhaustive diarrhœa, quickened respirations, mucous discharge, sometimes tinged with blood,

* Galtier, *Journ. de méd. vétér.*, 1889, p. 58 and 113; 1890, p. 625; Liénaux, *Ann. de méd. vétér.*, 1896, p. 625; Conte, *Rev. vétér.*, 1897, p. 516.

from the nostrils, and the special symptoms of broncho- and pleuro-pneumonia. The skin and visible mucous membranes present a more or less vivid red colour, at times punctiform hæmorrhages. Abortion is often observed, although the mother does not necessarily succumb to the disease. The intensity of the disease varies; it is sometimes so severe that death occurs in a few hours or days, and again the attack may be so slight as to be hardly perceptible. Convalescence from the severe forms is always prolonged.

Galtier further states that pneumo-enteritis (or swine fever) which is generally considered to be peculiar to the pig, extends to all farm animals, especially to the sheep, bovines, and solipeds. The disease being transmitted to the fœtus, calves coming from diseased cows which are or have been subject to coughing, are born with the germ of the disease in them, and die in a few days with the lesions of broncho-pneumonia and enteritis (pneumo-enteritis of calves). Whether this conclusion is correct is open to question, for though the bacilli of the septicæmia hæmorrhagica group are very similar, the predilection of the different organisms varies for different animals.

BACILLUS FELIS SEPTICUS.

This bacillus was isolated regularly by Fiocca from the saliva of cats.

Microscopical Appearances.—Very small short rods, 0.2 to 0.3 μ thick, often occurring as diplococci, and not producing spores.

Staining Reactions.—Stains by the ordinary methods, but not by the Gram method.

Biological Characters.—Its growth is similar to that of the rabbit-septicæmia bacillus.

In Bouillon it forms no flakes.

Milk is not coagulated.

On Potatoes a very thin, almost invisible coating.

Sugar-Media are not fermented.

Pathogenesis.—Produces septicæmia in mice, rabbits, guinea-pigs, and young rats.

BACILLUS TYPHI MURIUM.

This bacillus was discovered by Löffler in an epidemic among mice, the disease being named Mouse Typhoid.

Microscopical Appearances.—Small rods, which often form long threads.

Motility.—Actively motile.

Staining Reactions.—Easily stained with the ordinary stains, but not by the Gram method.

Biological Characters.—On *Gelatine Plates* the deep colonies are small, round, granular, and of yellowish-brown colour; the superficial colonies are flattened, irregularly notched, and possess delicate furrows similar to the colonies of the typhoid bacillus; the colonies are, however, more granular and of more luxuriant growth.

In Stab-Cultures.—Nail-like growth with a flat top.

On Agar, Blood-Serum, and Bouillon the growth is not characteristic.

On Potatoes the growth is moderate, a whitish coating being formed, the surrounding medium acquiring a dirty greyish-blue colour.

In Bouillon containing sugar, gas is formed.

Milk is not coagulated.

Pathogenesis.—Very virulent for white mice, house, and for field-mice, both by subcutaneous injection and by feeding. The infection is spread by the living mice eating the bodies of those dead of the disease. Löffler (*Centralbl. f. Bakter.*, xii., 1892), owing to this circumstance, used the cultures in destroying these animals during the mouse-plague in Thessaly.

SWINE FEVER.*

(Pneumo-enteritis; Hog Cholera; *Ger.* Schweinepest.)

History.—This disease was described in 1862 by Simmonds in England. Brown observed an outbreak in Berkshire in 1864. At the request made in 1878 by several counties, swine fever was scheduled as a contagious disease.

According to the Reports of the United States Bureau of Animal Industry, the disease first appeared in 1833 in Ohio, in 1858 in Illinois, Ohio, Indiana, Pennsylvania, New York, and Maryland—33 to 80 per cent. of all the pigs succumbing to the disease.

* Salmon and Smith, *Reports of the Bureau of Animal Industry*, Washington, 1886, 1889, 1894, 1897. Billings, *University of Nebraska Bulletin*, vol. ii., 1888; *Bulletins*, 7, 8, 9, 10, 1889.—Schütz, *Arch. f. wiss. u. pr. Thierheilk.*, 1886 and 1888.—MacFadyean, *Journ. of Compar. Pathol.*, 1895.—Kitt, *Bakterienkunde und pathologische Mikroskopie*, Wien, 1899.—Ostertag, *Handb. d. Fleischschau*, Stuttgart, 1899.—Annual Report of the Board of Agriculture, 1896, etc.

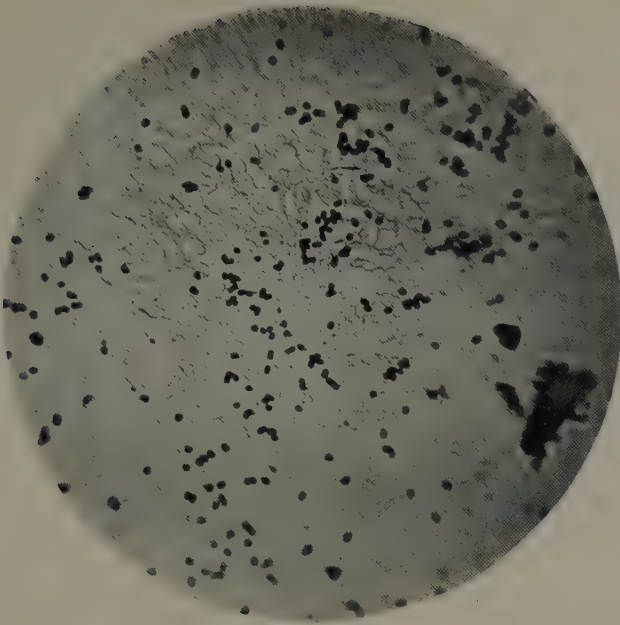


FIG. 93.—Bacillus of Swine Fever, with flagella. Agar culture. Stained by Bowhill's method. $\times 1000$.

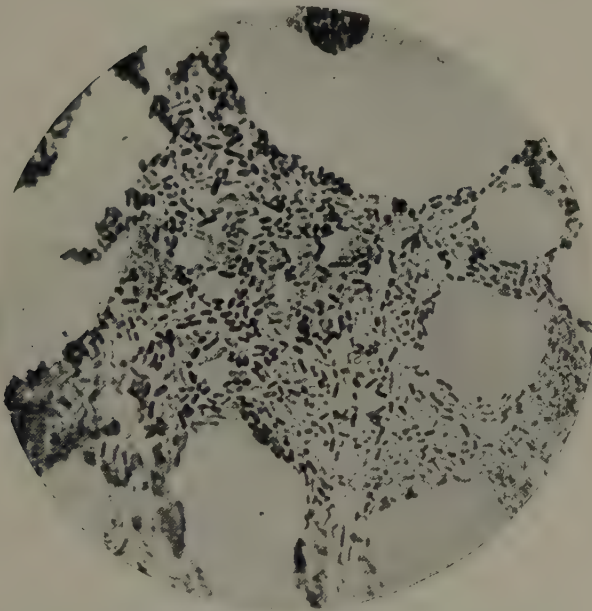


FIG. 94.—Bacillus of Swine Fever. Pure culture from pig's lymph-gland. Stained with methylene-blue. $\times 1500$.

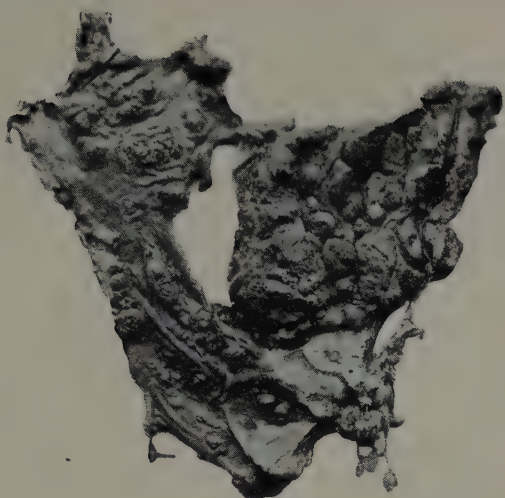


FIG. 95.—Stomach from pig dead of swine fever, showing ulcerations



FIG. 96.—Broncho-Pneumonia suis, lung of pig dead of swine fever.

Several varieties of hog cholera bacilli have been described. In 1890, Smith described a variety that was more saprophytic than the form usually found, and in 1894 he described seven varieties of the bacillus isolated from swine. These varieties differ either in their morphology, growth on media, the quantity of gas produced in glucose-bouillon, or their pathogenesis for rabbits.

In 1898, MacFadyean stated that swine fever in England was due to a specific bacillus, which when fed in pure cultures to healthy swine produced typical ulcerations such as are found in the intestines of pigs affected with swine fever contracted in the ordinary way. His description accorded in the main with the germ we are considering.

The chief veterinary officer, in his report to the Board of Agriculture for 1896, states: "It is quite certain that the disease which exists among the swine in America, where it has received the name of hog cholera, is identical with our swine fever, because in the year 1879 some cargoes of pigs affected with hog cholera were landed at Liverpool, when an opportunity was afforded of identifying the lesions of that disease with swine fever." These conclusions are identical with those published by the writer in 1891 regarding cultures obtained from an outbreak of swine fever in England, and sent to Billings in America, who concluded from his experiments that "It is the same germ as we have here as the cause of hog cholera."

Microscopical Appearances.—Short bacilli 1.2 to 1.5 μ long, and 0.6 to 0.7 μ broad, which do not form spores.

Motility.—Actively motile; possessing numerous flagella (see Fig. 93), like the *Bacillus typhi abdominalis*.

Staining Reactions.—Stains easily with any of the usual anilin dyes, but not by the Gram method. When freshly obtained from an animal, the bacilli exhibit bipolar staining (see Fig. 94), this being less evident in older cultures, or if the staining process is continued too long. In sections of organs the bacilli are seen to be present in the capillaries and small veins.

Spore Formation absent.

Vitality.—The bacillus is destroyed by a temperature of 58° C. in from fifteen to twenty minutes. It preserves its vitality in spite of desiccation for nearly two months. It retains its vitality for more than

fifteen days in sterilized water. It grows at a temperature such as prevails in summer.

On Gelatine Plates the surface colonies are spreading, round, or with irregular borders; the deeper colonies are spherical, small, almost homogeneous, and of a brownish colour.

Stab-Cultures in Gelatine resemble a nail with a flat top (see Fig. 100).

On Agar a greyish-white transparent growth occurs, and gas is formed in *Grape-Sugar Agar*.

On Potatoes a yellowish growth occurs.

Bouillon becomes cloudy, and a film forms on the surface.

Milk remains unchanged, and the reaction alkaline.

Neither *Indol* nor *Phenol* are formed.

Pathogenesis.—A small quantity of a culture kills rabbits and mice in seven to twelve days, the animals suffering from febrile symptoms. At autopsy the spleen is found to be enlarged and of a dark red colour. The liver exhibits necrotic patches. The kidneys are inflamed, and the urine contains albumen. The substance of the heart is flabby, and fatty degeneration is present. The mucous membrane of the small intestine is swollen, and ecchymoses are often present here and in the duodenum. The bacilli are present in small masses in all the organs, mostly in the capillaries and small veins. Injection of the virus into the lungs causes pneumonia. Feeding and inhalation of cultures also cause infection. Pigeons are somewhat refractory, but can be infected by large doses. Chickens are not infected, even with large quantities of the virus. Swine are somewhat refractory to subcutaneous inoculation, but by intravenous injection of 1 to 2 c.c., and by feeding with cultures, a severe diphtheritic inflammation of the stomach and large intestine is produced. In some cases the bacilli are less virulent, especially when obtained from chronic cases of swine fever. The character of the culture may also vary. Necrosis of the liver is not present in the milder forms of the disease, the changes in the bowel being more pronounced. Abscesses sometimes develop under the skin at the point of inoculation. Swine fever sometimes occurs in devastating epizootics, especially in America, when 90 per cent. of the swine may die. It occurs in an acute or hæmorrhagic septicæmic form, killing the animal in a few days; and a chronic form, in which case the disease may last two to four weeks, or even longer.

The lesions found at autopsy vary according to the duration and severity of the disease. Tumefactions occur around the head and back; the tongue is dark, and small necrosed patches and ulcers are often present on the lips, gums, and tongue. Numerous dark reddish-blue blotches, terminating diffusely in the surrounding tissue, are observable upon the abdomen. On cutting through the skin, dark blood escapes from the bloodvessels, and the abdominal cavity usually contains a large quantity of straw-coloured lymph with numerous flocculi floating in it. The large intestines are sometimes agglutinated by bands of flocculent lymph, the adhesions being very resistant in some parts. The mesenteric bronchial and superficial and deep inguinal lymph glands are usually enlarged, and on section present a greyish-red striated appearance. The mucous membrane of the large intestine is usually red and swollen, the ileo-cæcal valve being swollen, and often the seat of extensive ulcerations.

The small intestine usually presents a reddish appearance, the internal surface being studded with numerous dark red spots. The mucous membrane is swollen, and exhibits diffused capillary redness. The Peyer's patches are enlarged, and the contents of intestines fluid. The characteristic ulceration mentioned in connection with the ileo-cæcal valve may also be present, and, although involving the whole thickness of the bowel, perforation is extremely rare.

The liver is usually enlarged, the edges being rounded. On section dark blood exudes. The acini are considerably enlarged, and occasionally centres of necrosis have been observed. The gall bladder is usually distended, and full of viscid, dark greenish-yellow gall. The spleen is sometimes enormously enlarged and the pulp is slightly disintegrated. The trabeculæ are thickened, the Malpighian corpuscles enlarged, and sometimes a few white spots are seen upon the capsules. The stomach is sometimes the seat of numerous ulcerations (see Fig. 95); in some cases the cardiac portion is healthy, whilst the pylorus is congested and ulcerated. The kidneys are sometimes enormously swollen, and in a state of hæmatogenous nephritis, the pelvis in some instances being almost entirely occluded with blood-clots. Sometimes the kidneys are in a state of parenchymatous degeneration, and small petechiæ may also be observed under the capsule, which may or may not be adherent. The bladder sometimes contains bloody urine, this depending on the condition of the kidneys.

A wide difference of opinion exists among experts, as to whether pneumonia is or is not a diagnostic symptom of swine fever. According to MacFadyean (1899) the pig may be the subject of a genuine swine fever pneumonia. The thoracic lesions observed in genuine cases of swine fever are: slight effusion into the thorax, and at times attachments or adhesions of the pleuræ. Small ecchymosed spots are sometimes found on the pleura costalis pulmonalis, and also on the pericardium. The pericardial sac may be distended through an enormous quantity of bright yellow fluid, and the visceral folds studded with numerous bright red ecchymosed spots. The lungs are sometimes marked by numerous red or reddish or blue-black spots of varying size, some of which extend beyond the surface of the lung, and often correspond to a single lobule; large centres of hepatization are also often present (see Fig. 96). On section some lungs reveal a condition of lobular broncho-pneumonia, the

bronchial mucosa being swollen, and the bronchi containing a yellowish-red exudate.

According to Bang, the pneumonic and intestinal lesions that occur in chronic cases of swine fever are not caused by the swine fever bacillus, but by the "Schweineseuche" bacillus, which he designates the "Vacuole" bacillus. This organism is present in the lungs and nasal secretion of healthy swine. Associated with the "Vacuole" bacillus, Bang also found the "Necrosis" bacillus, this organism being occasionally found in the intestines of healthy swine. Bang's conclusions are corroborated by the researches of Preisz, Karlinski, and Ostertag.

Immunity.—Billings obtained protection against the disease by inoculating swine with cultures derived from mild cases of the natural disease, but a certain proportion of the animals died from the effects of the inoculation. Smith claims to have obtained better results by injecting small doses of the virus intravenously. De Schweinitz succeeded in immunizing guinea-pigs by means of soluble substances from cultures, and has recently produced immunity by means of an anti-toxic serum obtained from the blood of immunized animals. A number of guinea-pigs were treated with serum from a pig rendered immune to hog cholera, the doses of serum varying from 0.5 to 4.5 c.c. Fifteen days later these guinea-pigs resisted the otherwise deadly injection of 1 to 10 c.c. of a one-day-old hog cholera culture. In another experiment, three guinea-pigs, eight months after being immunized, still remained capable of resisting the above-mentioned fatal dose. The immunity did not continue for a longer period, because the serum experimented with was obtained from a pig immunized a long time before. It therefore appears that the anti-toxic substance is only present free in the blood, and capable of conferring immunity on other animals, immediately after the animal is immunized. De Schweinitz also produced immunity against Schweineseuche with anti-toxic serum, but it was not possible with the Schweineseuche serum to produce immunity against hog cholera, or *vice versa*. According to Salmon, the products of the hog cholera germ are very irritating, and when injected into the tissues they are liable to cause the formation of large abscesses which hinder the absorption of the cultures into the general circulation, thus preventing the animal from acquiring immunity. The serum used by the U.S. Bureau in 1897 saved over 80 per cent. of the animals when used in affected herds. The methods of making

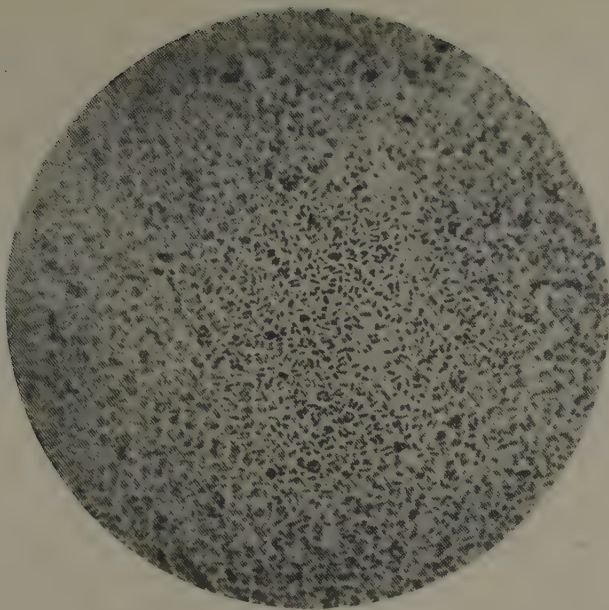


FIG. 97. --*B. suisepicus*. Agar culture. $\times 1000$.

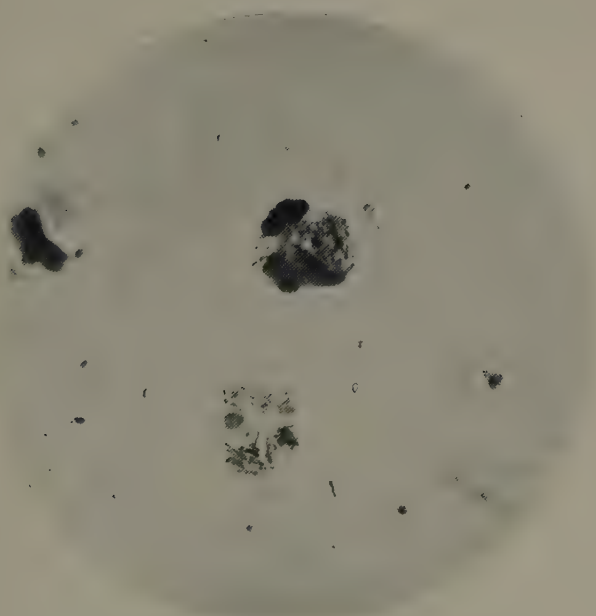


FIG. 98. --*B. murisepticus* in mouse's blood. Stained by Claudius method. $\times 1000$.

the serum have now been considerably improved, and it appears that a serum of higher efficiency will be obtained. There is no danger in using this serum, as it is absolutely free from the germs of the disease. It is easily applied, and the good effects in sick hogs are seen almost immediately. It remains to be tested on a larger scale before absolute assurance can be given.

BACILLUS SUISEPTICUS.*

(Swine Plague ; Infectious Pneumonia of the Pig ; *Ger.* Schweineseuche.)

This bacillus was first described by Löffler and Schütz, and is also mentioned by Smith as being associated with his hog cholera germ, as well as occurring alone.

Microscopical Appearances.—Short bacilli, morphologically and in cultures very like the chicken cholera bacillus. (See Fig. 97.) It does not form spores, and is non-motile.

Staining Reactions.—Stains easily with the ordinary anilin dyes, but not by the Gram method. Bipolar staining is well-marked in young cultures.

Biological Characters.—*On Gelatine Plates* the deep colonies are round and of brownish colour, the surface growth being limited.

In Gelatine Stab-Cultures the growth resembles a nail with a flat top. (See Fig. 99.)

On Agar it forms a thin coating with crenated margins.

On Potatoes it does not grow except when they have been rendered alkaline, and then it forms a yellowish covering.

Bouillon remains clear, but a thick sediment is formed.

Milk is not coagulated, but a weak acid reaction is produced. Indol is formed.

Pathogenesis.—Rabbits, small birds, and mice are as easily infected as with chicken cholera, and generally die in twenty-four hours from septicæmia. Guinea-pigs are not so easily affected, but young ones die quickly. In all these animals the effects produced at the point of inoculation are more intense than with the bacillus of chicken cholera (extensive hæmorrhagic œdema). The liver frequently

* Löffler and Schütz, *Arb. a. d. Kais. Gesundheitsamte*, Bd. i., 1886; Frosch, *Zeitschr. f. Hygiene*, Bd. ix., 1890; Smith, *ibid.*, Bd. x., 1891; Smith, *Centrabl. f. Bakt.*, Bd. ix., 1891, Bd. xvi., 1894.

shows fatty degeneration. Chickens are affected by large doses. Subcutaneous injection in swine produces marked œdema at the point of inoculation, and a fatal septicæmia. When injection into the thorax is practised, pigs die from a multiple necrotic pleuro-pneumonia, the bacilli being present in the blood; there is also a slight enlargement of the spleen, and catarrh of the mucosa of the stomach. Infection does not result by feeding. Calves likewise succumb from subcutaneous inoculation with *Bacillus suissepticus*. The disease consists essentially of a pleuro-pneumonia, with inflammatory necrosis, and when the process becomes chronic caseous deposits occur. The caseous deposits are also found in the large intestines, and adjoining mesenteric lymph-glands.

BACILLUS OF SWINE ERYSIPELAS AND MOUSE SEPTICÆMIA.*

(*Ger.* Schweinerothlauf; *Fr.* Rouget du porc. *Bacillus rhusiopathiæ*, Kitt; *B. murisepticus*.)

This disease is peculiar to the pig, chiefly affecting adult animals, the improved breeds being more predisposed. The characteristic symptoms are great pyrexia, the appearance of red or purple blotches upon the skin—these being at first limited, but afterwards confluent. In very acute cases the redness of the skin may not be present. The animals suffer from constipation followed by diarrhœa. The average duration of the disease is about two days, but it may last four or five days, the fatality being sometimes as high as 60 per cent. Most authors agree that this bacillus is identical with Koch's *B. murisepticus*.

Microscopical Appearances.—A very fine bacillus, 1 to $1\frac{1}{2}$ μ long, and 0.2 to 0.6 μ broad, morphologically identical with the bacillus of mouse septicæmia. The bacillus is found in the blood, especially in fine capillaries, in contact with their internal wall. It is also present in the exudates, in all the diseased organs, in

* Pasteur and Thuillier, *Bull. de l'Acad. méd.*, Paris, 1883.—Klein, *Virchow's Arch.*, Bd. xcv.—Löffler, *Arch. a. d. Kaiserl. Gesundheitsamte*, 1885.—Loir, *Ann. de l'Inst. Pasteur*, 1887.—Schottelius and Lüttin, *Der Rothlauf der Schweine, seine Entstehung und Verhütung*, Wiesbaden, 1885.—Kitt, *Bakterienkunde u. patholog. Mikroskopie*, p. 384.—Kitt, *Jahresb. der Thierärztl. Hochsch.*, München, 1887; *Centralbl. f. Bakteriöl.*, Bd. xi., 1887.—Chamberland, *Ann. de l'Inst. Pasteur*, 1894, p. 161.—Emmerich, *Münchener med. Wochenschr.*, 1891.—Lorentz, *Centralbl. f. Bakt.*, 1894, Bd. xv.; *Deutsche Zeitschr. f. Thiermedizin.*, Bd. xx., xxi.



FIG. 99.—Bacillus of Schweineseuche. Gelatine stab-culture.

FIG. 100.—Bacillus of Swine Fever. Gelatine stab-culture.

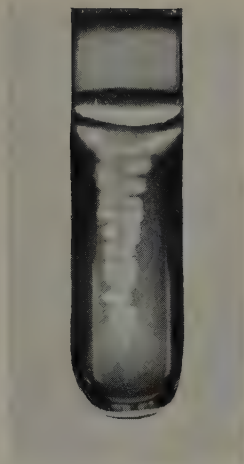


FIG. 101.—Bacillus of Swine Erysipelas. Gelatine stab-culture.

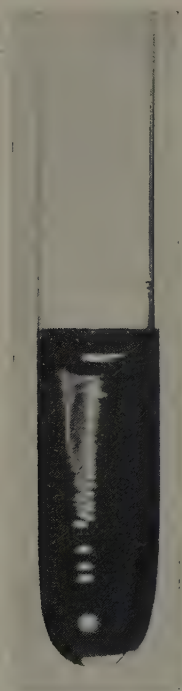


FIG. 102.—Bacillus of Mouse Septicæmia. Gelatine stab-culture.

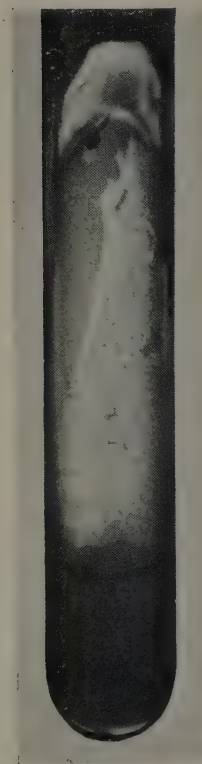


FIG. 103.—Bacillus of Bubonic Plague. Oblique agar culture.

the marrow of the bones, in the fæces, and in the urine. Sometimes the leucocytes are filled with bacilli. (See Fig. 98.)

Motility undetermined (Günther).

Staining Reactions.—Stains best with fuchsin, and beautifully by the Gram and Claudius methods, especially in sections of organs.

Biological Characters.—Spores are not formed, but involution or degenerate forms are frequent. Drying weakens the bacilli quickly, but they can live a long time in filthy fluids, and the bacilli, according to Kitt, are very difficult to kill in large pieces of meat, such as hams, by cooking, pickling, salting, or smoking. Koch, it will be remembered, first isolated *B. murisepticus* from putrid blood.

On Gelatine Plates small transparent, slightly liquefying colonies are formed, which under a low power resemble a mass of threads.

In Gelatine Stab-Cultures the growth occurs along the track of the needle, with numerous ramifying out-growths into the surrounding medium, giving the growth the appearance of a test-tube brush. (See Fig. 102.)

On Agar growth occurs as a fine coating.

On Potatoes no growth.

In Bouillon slight cloudiness, with the formation of a sediment.

Indol is not formed.

Pathogenesis.—Affects white and grey mice, white rats, rabbits, and pigeons. The animals generally die in three to four days, sometimes later; the day before they die, they sit motionless in one place, their eyes being firmly closed with secretion, and their heads retracted as if they were sleeping, and in this position they die. Animals can also be infected by feeding; but rabbits are less susceptible to this mode of infection. Intravenous injection kills rabbits in three to six days. The field and wood mouse, guinea-pig, ox, horse, ass, dog, cat, chicken, goose, and duck are immune. Sheep seem to be more disposed to infection.

The result of experimental infection in swine varies according to the breed; well-bred swine die from cutaneous inoculation and feeding with infectious material.

Post-Mortem Appearances in Swine.—The skin is œdematous and infiltrated with blood; the flesh is soft, greasy, and of a pale red

colour. The lymph-glands, particularly those of the mesentery, are swollen and infiltrated with blood, presenting a red streaky appearance due to engorgement and distension of the bloodvessels of the gland. There is sero-fibrinous exudation from the pleura and pericardium. The peritoneum is congested and covered with ecchymosed spots. The mucous membrane of the bowel is highly congested and swollen, and in many places the epithelium is desquamated, and occasionally partially-formed ulcers are present. The bacilli are widely distributed, but not so plentiful in the blood as in experimentally infected animals. According to Schottelius another bacillus is sometimes found associated with the erysipelas bacillus in the organs. This organism, the *Bacillus coprogenes fetidus*, is found in the contents of the bowel and neighbouring organs of animals affected with erysipelas. It is non-motile, shorter than the *Bacillus subtilis*, and forms spores. The cultures emit a putrid odour. The presence of this organism in animals affected with erysipelas is of no etiological importance. Some of the mild affections of swine, such as urticaria or nettle-rash, skin necrosis, and endocarditis verucosa bacillosa, are considered by Kitt, Bung, and Jensen to be caused by the bacillus of swine erysipelas. The fæces of affected swine are very virulent. The disease is spread by means of rats and mice.

Immunity.—The repeated passage of the erysipelas bacillus through the pigeon renders it more virulent for swine, whilst its passage through the rabbit, on the contrary, diminishes its virulence for swine. After a time the virus obtained from the rabbit does not kill swine, but produces a mild attack, which renders them immune to the action of the virulent germ. The degree of attenuation obtained persists in cultures made in ordinary bouillon, which is used as a vaccine for swine.

Pasteur's method consists in using two vaccines of different degrees of virulence in doses of 0.12 c.c., an interval of ten days intervening between the first and second inoculation. As young pigs are not so susceptible to the disease as older ones, they are more suitable for immunization. Swine vaccinated by this method remain immune for one year, which is long enough for breeding and fattening purposes.

Lorenz has introduced a method of protective inoculation with the blood-serum of immunized swine. It is very difficult to procure a serum of uniform strength in swine erysipelas, because it is not easy,

and often impossible, to obtain a uniform grade of virulence in swine erysipelas cultures. The serum is injected subcutaneously behind the ear or between the thighs.

DIFFERENTIAL DIAGNOSIS TABLE.

	SWINE ERYSIPELAS.	SWINE FEVER.	SCHWEINESEUCHE.
(1) Size of the organism	1 to 8 μ long; 0.2 to 0.6 μ broad	0.6 to 1 μ long; 0.4 μ broad	0.6 to 1 μ long; 0.4 μ broad
(2) Motility	Non-motile	Actively motile flagella numerous	Non-motile
(3) Staining	With ordinary stains and by the Gram and Claudius methods	Exhibits polar staining with the ordinary anilin stains; negative with the Gram and Claudius methods	As in swine fever
(4) Cultures	Characteristic brush-like growth in gelatine stab-cultures; no growth on potatoes	Grows on agar and in gelatine stab-cultures, nail-like form, also on potatoes, forming a yellow coating	The growths on agar and gelatine resemble swine fever, but there is no growth on potatoes unless they are alkaline, when a yellowish coating is formed
(5) Indol reaction	Absent	Absent	Present
(6) Gas-formation	None	Present	None
(7) Inoculation experiments	Mice die in two to four days, rabbits in four to six days. Guinea-pigs and chickens are immune, and swine over three years of age are not affected	Rabbits and mice die in seven to twelve days; large doses fatal to pigeons; chickens are immune. Swine difficult to infect by inoculation. Feeding experiments yield more positive results	Rabbits, small birds, and mice generally die in twenty-four hours from septicaemia; young guinea-pigs easily killed, but not so older ones; chickens affected by large dose. Feeding experiments negative, but infection by inoculation positive in swine

THE BACILLUS OF BUBONIC PLAGUE.

B. pestis.

The organism causing this disease was discovered simultaneously by Kitasato and Yersin in the outbreak of bubonic plague at Hong-Kong in 1894.

Microscopical Appearances.—Short oval bacilli with rounded ends, usually occurring singly, in pairs, or short chains; very often enclosed in a capsule.

Motility.—Slightly motile ; Gordon has demonstrated the existence of one to two flagella.

Spores are not formed.

Staining Reactions.—Exhibits polar staining with the ordinary staining methods (see Figs. 104 and 105), but does not stain by the Gram method.

Biological Characters.—The bacillus grows at 18°, but best at 36° to 39° C.

On Gelatine Plates small, dark, granular colonies of a brownish colour are formed ; the medium is not liquefied.

In Gelatine Stab-Cultures it develops slowly on the surface and along the track of the needle.

On Agar Plates, in twenty-four hours, small dewdrop-like colonies appear on the surface, which, in forty-eight hours, show slightly iridescent borders. The deep colonies are round and granular.

On slanted Agar a viscous, shiny coating appears ; where the growth is sparse, colonies like dewdrops may be observed. The water of condensation is clouded, but no film is formed. (See Fig. 103.)

The growth on *blood-serum* is similar to that on ordinary agar.

Bouillon becomes diffusely clouded, but if it is inoculated with a cohesive mass of bacteria from an agar culture, the bacilli develop on the bottom of the tube, while the upper portion of the medium remains clear ; this mode of growth being somewhat similar to that of streptococci. Haffkine lays stress upon the "stalactite" appearance of the growth near the surface.

Milk is not coagulated, though acid is formed.

On Potatoes, at 37° C., a scanty, greyish-white growth occurs on the surface.

The bacillus of bubonic plague forms *gas* in sugar-containing media, and no *indol* in either bouillon or peptone water. It grows best on media of neutral reaction (Wladimiroff and Kressling).

Vitality.—The plague bacillus is killed by heating for ten minutes at 55° C., and five minutes at 80° C. Corrosive sublimate 1-1000 destroys the bacilli immediately ; 1 per cent. carbolic acid, or 1 per cent. lysol, kills the bacillus in ten minutes. The mineral acids are very effective : sulphuric acid 1-2000 kills the bacillus in five minutes ; hydrochloric acid 1-1000 in thirty minutes. The longest time that

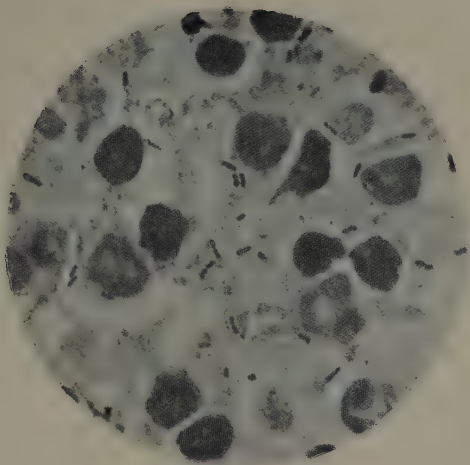


FIG. 104.*—*B. pestis*. Cover-glass specimen from spleen of inoculated mouse. Methylene-blue. $\times 1000$.

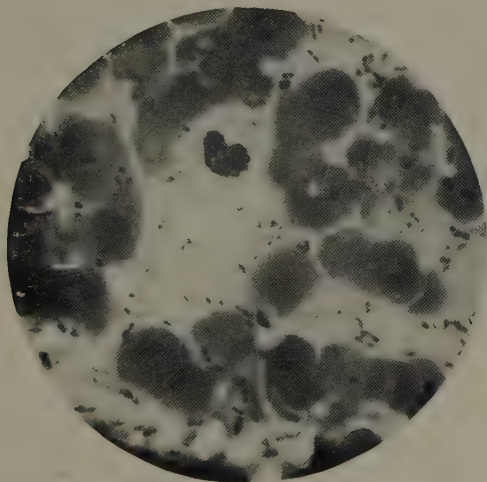


FIG. 105. —*B. pestis* in human blood. $\times 1000$.

* I am indebted to Dr G. H. F. Nuttall for the specimen from which this photograph was taken.

infected material on lint, wadding, earth, etc., remained active was eight days. Sputum (from patients affected with the pneumonic form), which was kept in a vessel plugged with cotton wool, was no longer virulent after sixteen days. In drinking water the bacillus dies in three days; in sterilized water in eight days; in sterilized bilge-water in five days. Direct sunlight kills the bacillus in three to four hours. The bacilli die in four days when dried and maintained at ordinary room temperature.

Pathogenesis.—Rats and mice of all species are most susceptible, a minimum quantity of virulent culture being sufficient, when injected subcutaneously, to cause death. The same results are obtained when the virus is rubbed upon the mucous membrane of the nose or eye. Infection also occurs through feeding, the animals infecting themselves by gnawing their companions that have died of plague. The latter mode of infection is of great importance, as it explains the rapidity with which the disease spreads amongst rats.

Guinea-pigs, rabbits, squirrels, pigs, monkeys, cats, chickens, and sparrows are susceptible to experimental infection, and some of these animals have been observed to acquire the disease under natural conditions. Nuttall has shown that lizards and snakes are susceptible when kept at a temperature of 20° C. Dogs, cattle, hedge-hogs, and geese are immune.

Yersin observed the bacillus in the bodies of dead flies in the plague laboratory at Hong-Kong, and Nuttall found that flies died when fed with plague tissues (in seven to eight days at 12° to 14° C., in about three days at 23° to 31° C.). The bacilli were soon digested in the bodies of bed-bugs. The bacilli have been repeatedly found in the bodies of fleas taken from rats and mice dead of plague.

In animals subcutaneously inoculated, the seat of inoculation becomes œdematous, and the lymphatics enlarged in a few hours; in twenty-four hours the animal is quiet, the hair is rumped, there is excessive lachrymal discharge from the eyes; finally convulsions set in, and death supervenes. The post-mortem appearances are as follows:—Bloody œdema at the point of inoculation, reddening and swelling of the lymph-glands; hæmorrhagic extravasation into the abdominal walls; serous effusion into the thoracic and abdominal cavities; intestines congested. The spleen is enlarged, and greyish points resembling miliary tubercles are sometimes present. The suprarenal capsules are

enlarged. The characteristic bacilli can be found in large numbers in the local œdema, lymph-glands, blood, and internal organs.

In man, when the virus gains entrance through the skin of the lower extremities, the superficial and deep inguinal glands are the first to become affected. If the infection be through wounds in the hands, then the buboes appear first in the axillary region. A member of the German Plague Commission was infected while performing an autopsy on a victim of plague; two days later a small pustule appeared on the right hand, and this was soon followed by lymphangitis and enlarged axillary glands—the plague bacilli being found in the secretion from the pustule. Infection also occurs through inhalation, large numbers of bacilli being found in the sputum in pneumonic plague.

Bacteriological Diagnosis of Plague.—As already mentioned, the characteristic bacilli are very numerous in the pustules and buboes, and the polar staining exhibited with methylene-blue enables them to be easily differentiated. The bacillus of chicken cholera resembles it, but is somewhat larger, and has no effect on man. The diagnosis is not so easy in those cases where suppuration has not commenced. The German Commission consider puncture of the bubo somewhat dangerous, on account of opening bloodvessels, but the English doctors make a long incision into the affected gland, which is afterwards dressed with antiseptics. By this method it is easy to obtain material from the gland for cover-glass specimens and cultures. Microscopical examination of the blood only yields positive results in cases of general infection (septicæmia). Cultures from the blood are more apt to yield positive results. To isolate plague bacilli from sputum, it is best to make gelatine stroke plate cultures. The suspected material is drawn across the surface of solid gelatine, several strokes being made. The plague bacilli grow very well at 22° to 25° C., while the associated bacteria, *i.e.*, *Diplococcus lanceolatus*, *Streptococcus*, etc., generally exhibit only a scanty growth. According to Hankin, when the bacilli are grown on agar (containing 2.5 to 3.5 of salt) at 31° C., involution forms, consisting of pear-shaped bodies and spheres, appear after twenty-four to forty-eight hours, and he considers these characteristic enough for diagnosis. The blood-serum of men and animals who have recovered from or have been immunized against plague, possesses the power of *agglutination*. The agglutinating effects of serum may also be observed during the second and third weeks of illness. In

the cases of mixed infection, particularly those accompanied with suppuration of the buboes, *Streptococci* may be found in the glands and blood.

Immunity.—An anti-plague serum has been prepared from immunized horses at the Pasteur Institute. The German Commission, experimenting with this serum, found that 3 c.c. was sufficient to protect a brown monkey against a subsequent subcutaneous inoculation. With the susceptible grey monkey 10 c.c. of serum was not sufficient to prevent the disease. The serum exhibited undoubted curative effects on a brown monkey inoculated twelve hours previously. Haffkine prepares his plague prophylactic from fluid cultures rich in fat. The development is allowed to go on for about a month, the broth being shaken now and then to ensure a series of successive crops. The organisms are then killed by exposure to 70° C. for an hour. Before use the dead culture is shaken, and 3 c.c. are injected.

BRONCHO-PNEUMONIA BOVIS.

(Infectious Broncho-pneumonia; Corn-Stalk Disease.)

This disease occurs among American cattle. It is caused, according to Billings, by an ovoid belted organism which infects cattle eating the leaves and tender top shoots of corn stalks, and from this circumstance he named it "The Corn-stalk Disease." Nocard, on the other hand, found some cattle amongst a lot landed from America affected with a specific lung disease, which at the first glance presented the appearance of recent lesions of contagious pleuro-pneumonia; but careful microscopical examination revealed the presence of ovoid belted organisms that could be observed in the lymph-spaces in pure culture. The author discovered the presence of morphologically identical organisms in sections of a piece of the lung of an American ox received from Professor Williams.

Microscopical Appearances.—Short oval bacteria 1 μ long, and about .5 μ in width.

Motility.—Actively motile.

Staining Reactions.—Stains readily with alkaline methylene-blue, and shows clear spaces in the centre of the organism. The bacillus does not stain by the Gram method. Sections are best stained with

methylen-blue, according to Weigert's method (see p. 41), or with fuchsin.

Biological Characters.—According to Billings the growth is more characteristic at room temperature than in the incubator.

On Gelatine Plates, flat, spreading, bluish, transparent, lobulated colonies develop.

In Gelatine Stab-Cultures the growth takes place along the track of the needle and on the surface of the medium; there is no liquefaction.

Upon slanted Gelatine there is a rapid, pellucid, pearly-white growth, the edges being scalloped.

On Agar the edges of the growth are deeply scalloped.

On Potato, greyish-white, somewhat elevated colonies develop.

On White of Egg, clear yellow colonies with slightly raised edges develop.

Milk is not coagulated.

Pathogenesis.—Mice, rabbits, guinea-pigs, and pigeons inoculated subcutaneously with 2 or 3 drops of culture die in less than forty-eight hours. Intraperitoneal injection causes death in fifteen to eighteen hours with purulent peritonitis. Sheep and calves inoculated subcutaneously, or in the trachea, with 1 c.c. of culture, serous exudation, or virulent pus, did not die, but were seriously affected for several days, after which they soon regained their normal condition. An eight-month-old calf and a two-year-old ram, inoculated in the right lung with 5 drops of peritoneal pus from a guinea-pig, died in less than forty-eight hours with fibrinous pleurisy and exudative bronchopneumonia analogous to that observed at the autopsy on the American cattle. The lesions contained quantities of bacteria (Nocard). Pigs, dogs, rats, and chickens are immune.

BACILLUS OF CANINE DISTEMPER.

(*Febris catarrhalis epizoötica canis.*)

The bacillus of canine distemper, isolated by Jess, was obtained by means of plate cultures, prepared from the nasal discharge of an affected dog, and incubated at 37.5° C. When the cultures were examined twenty-four hours later, various shaped colonies were detected microscopically, and amongst these, prominent, granular, dark-coloured, whetstone-shaped colonies were observed, especially

in cultures on gelatine media. This peculiar whetstone formation enables the distemper bacillus colonies to be easily distinguished from other associated colonies.

Morphology.—Very small rods 1.8 to 2.3 μ long (the latter dimension is only attained in the animal body), and 0.6 μ broad (in the body 0.9 μ). The bacilli are found in the nasal and conjunctival discharges, in the blood, peritoneal effusion, and also in the organs. They are motile.

Staining Reactions.—The bacillus can be stained with the ordinary dyes, as also by the Gram method. In smear-preparations from the conjunctival and nasal discharges, stained with carbol-fuchsin, the bacilli exhibit polar staining. In preparations from bouillon cultures the rod stains uniformly. Beautiful results are obtained when specimens prepared from the typical whetstone-shaped colonies are stained with a watery solution of gentian-violet (heating the specimen during the staining process until vapour arises). The bacilli in agar and gelatine cultures exhibit polar staining.

Growth.—In cultures kept at 15° to 16° C., the typical whetstone-shaped colonies develop in about three days.

On Agar a pale grey coating develops in twenty-four hours at 37° C. The edges of the growth are serrated, and the condensation water clouded.

On Glycerine Agar the growth is scanty, the colonies being mostly isolated.

On Gelatine Slants an abundant growth takes place.

In Bouillon, clouding of the medium occurs in twenty-four hours, a fine thready coating forming on the surface, whilst on shaking the tube a flaky sediment rises from the bottom.

On Potatoes, at 37° C., a white, velvety growth develops in forty-eight hours.

On Blood-Serum Agar a brownish coating, with sharp, light greyish edges, develops slowly.

Pathogenesis.—A terrier seven weeks old was inoculated intrapleurally with 3 c.c. of a bouillon culture several days old. After five days it had extensive (watery) nasal discharge and adhesion of the eyelids. A cat which had been under observation for several weeks, to be certain of its freedom from distemper, was placed in the

same cage as the sick dog. Four days later (nine days after the infection of the dog), the cat became ill, it did not eat, had discharges from the eyes and nose, which five days later became purulent. The inoculated dog recovered in fourteen days; the cat was destroyed, as its recovery was impossible. A mongrel dog was also inoculated subcutaneously on the inside of the thigh with a bouillon culture two days old. The right inguinal gland was enlarged, and a hot, painful, diffuse swelling appeared at the point of inoculation. An extensive lachrymal discharge appeared in four days, together with diarrhoea, the symptoms subsiding after seven days. An old healthy cat was placed in the cage. It became infected and recovered. Another cat was also inoculated on the inside of the thigh with a pure culture, and died in three days.

Galli-Valerio (1896) also described an "Oval Bacillus" associated with canine distemper. The bacillus measured 1.25 to 2.5 μ in length, and 0.3 μ across, and was found in the brain, lungs, and spinal cord of affected animals. The bacillus was pleomorphic, occurring either in pairs, lying side by side, or in long threads. It stained according to Gram. Rabbits and guinea-pigs were immune.

DIFFERENTIAL DIAGNOSIS TABLE.

Showing the difference between the Bacilli of Jess and Galli-Valerio.†*

GALLI-VALERIO'S OVAL BACILLUS.	JESS'S DISTEMPER BACILLUS.
<i>Rabbits</i> —Immune.	<i>Rabbits</i> —Great swelling at the point of infection; duration of sickness one week.
<i>Guinea-pigs</i> —Immune.	<i>Guinea-pigs</i> —Killed in thirty-six hours.
<i>White mice</i> —	<i>White mice</i> —Susceptible.
<i>Cultures</i> —	<i>Cultures</i> —
<i>Agar</i> —Small white points.	<i>Agar</i> —Pale grey coating with serrated edges.
<i>Potatoes</i> —White transparent coating.	<i>Potatoes</i> —Extensive white velvety coating.
<i>Form</i> —Oval bacilli; pleomorphic.	<i>Form</i> —Always rod forms.
<i>Staining</i> —Polar staining sometimes observed.	<i>Staining</i> —Polar staining with fuchsin in bacilli from the nasal discharge and old cultures.

* Jess, *Centralbl. f. Bakteriöl.*, Bd. xxv., p. 541.

† Galli-Valerio, *Centralbl. f. Bakteriöl.*, Bd. xix., p. 694.

BACILLUS AËROGENES CAPSULATUS.*

This organism was discovered by Welch in 1891, and first fully described and named by Welch and Nuttall in 1892, having been isolated from the blood and organs of a negro, who died of aneurismal hæmorrhage, the autopsy being made eight hours after death. The blood and organs were found to contain gas evolved by the bacillus. Since the appearance of the publication referred to a considerable number of cases have been observed in man, proving that this bacillus may, under certain conditions, exert a pathogenic action in man, chiefly in consequence of lacerated and gunshot wounds, which produce conditions favourable for the multiplication of the germ.

Welch, Howard, Hitschmann and Lilienthal, Hirschberg, and others, have shown the bacillus to be frequent in the fæces of man and a variety of animals, as also in the soil and dust. Schattenfroh and Grassberger, as also Hirschberg, have found it in market-milk.

Microscopical Appearances.—Straight or slightly curved rods with blunted extremities, measuring on an average 3 to 6 μ in length, provided with large capsules, and occurring singly, in pairs, clumps, and short chains. The bacillus is non-motile. According to Howard, the bacillus forms spores on blood-serum.

Staining Reactions.—Stains with the ordinary dyes and by the Gram method.

Biological Characters.—The bacillus is an *obligatory anaërobe*. It grows on all ordinary culture media at 18° to 37° C., best at body temperature and in media containing sugar.

In Agar it grows best when 1 per cent. glucose is added, showing rapid development within twenty-four hours at 37° C., the colonies being greyish or brownish-white, spherical or ovoid, irregular, showing hairy or feathery projections in older cultures. A large amount of gas (chiefly H) is formed, and the cultures emit an odour of stale cheese, as they also do in bouillon.

In Gelatine the colonies have the same appearance as in agar. The medium is not liquefied.

* See Welch and Nuttall, *Johns Hopkins Hospital Bulletin*, No. 24 (1892); Welch, *Centraltbl. f. Bakteriologie*, xxix. (1901), p. 442, etc.; a review of the literature is given by Love and Cary, *Medical Record*, lv. (1899), p. 493.

Bouillon is diffusely clouded, bubbles of gas form at the surface of the fluid ; an abundant sediment forms, after which the fluid becomes clear. Strong acid reaction developed.

Potatoes, when all oxygen has been removed, show a thin, moist, greyish-white growth upon the surface, much gas being formed in the fluid about the sides and bottom of the potato.

Vitality.—In sugar-bouillon in a hydrogen atmosphere the bacilli die in about three days. When the oxygen has been removed by Buchner's method, the bacilli live one hundred and twenty-three days and over in cultures. When spores have been formed, these remain alive indefinitely. The vegetative forms in bouillon are destroyed by ten minutes' exposure to 58° C.

Pathogenesis.—Doses of 2.5 c.c. of twenty-four hour bouillon culture injected into the ear-veins of normal rabbits produced no pathogenic effects. One pregnant rabbit, which was proved to have had two dead embryos in its uterus, was killed inside of twenty-one hours by a dose of 1 c.c. of culture, the blood and organs of the animal being filled with gas. The dead embryos and affected uterine tissues in this animal had served as a foothold for the gas bacillus, exactly as in fatal cases in man.

BACTERIA FOUND IN THE MOUTH.

The following have not as yet been successfully cultivated artificially :—

1. *Leptothrix buccalis innominata* forms manifold, twisted, and intertwined motionless threads, which are stained yellow with a solution of iodine and iodide of potash.

2. *Bacillus buccalis maximus* appears in small tufts running parallel. Stain blue-violet (Granulose reaction) with the iodine-iodide solution.

3. *Leptothrix buccalis maxima* in its form and arrangement presents a great resemblance to the *Bacillus buccalis maximus*, but is stained yellow with the iodine solution.

4. *Iodococcus vaginatus* is found in chains consisting of four to ten cells, which are enclosed in a sheath about 0.75 μ thick. The cells are stained a blue-violet with the iodine solution, while the sheaths are stained a pale yellow colour.

5. **Spirillum sputigenum** is comma-shaped and actively motile; when two germs remain adherent they appear like the letter S.

6. **Spirochæte dentium** (*Sp. denticola*).—This organism, like the above-mentioned, is found under the edges of the gums. It forms long spirals from 8 to 25 μ long, with sharp-pointed ends, resembling the Spirochæte of relapsing fever.

7. **Leptothrix gigantea**.—This organism was found by Miller in the coating of the teeth of a dog affected with Pyorrhœa alveolaris, and named gigantea on account of its great size.

BACTERIA FOUND IN URINE.

Micrococcus ureæ (Leube).—Cocci 0.8 to 1.0 μ in diameter, occurring either singly, in pairs, tetrads, or in chains. It grows on the surface of gelatine without causing liquefaction.

Micrococcus ureæ liquefaciens (Flügge), 1.25 to 2 μ in diameter, occurs either singly or in small chains or irregular groups. It liquefies gelatine slowly.

Bacillus ureæ (Leube), plump rod 1 μ wide, with rounded ends. Grows on the surface of gelatine without liquefying the medium.

Urobacillus pasteyri is found in putrid urine. It is motile and of varying length, and forms threads. Spores are formed at one end of the rod.

Bacillus glischrogenus is found in slimy urine. It is motile, and forms a slimy substance in urine, milk, and solutions of starch.

BACTERIA OCCURRING IN AIR, SOIL, AND WATER.

I.—BACILLI.

1. Which do not liquefy Gelatine.

(a.) CHROMOGENIC.

Bacillus aurantiacus.—Small thick rods, exhibiting slight motility.

On Plate Cultures superficial, button-shaped, orange-coloured colonies develop.

In Gelatine Stab-Cultures it exhibits a shiny orange-coloured growth.

In Bouillon the growth is very characteristic, the fluid remains clear, and a membrane with isolated orange-coloured specks forms on the surface.

Bacillus fluorescens non-liquefaciens occurs in the form of delicate, short, actively motile rods. Another form is described, the *Bacillus fluorescens nonliquefaciens immobilis*, which is distinguished by its non-motility. The colonies on gelatine possess a mother-of-pearl appearance, and greenish fluorescence.

On Agar the growth is greenish.

Bacillus fuscus occurs in the form of medium-sized rods, which are often curved. So named because of the dark brown coloured pigment it produces.

Bacillus rubefaciens.—Fine rods, two or more remaining united. The growth in *gelatine* possesses a pale rose-red colour.

On Potatoes the substratum is rose-coloured, while the colonies themselves vary in colour from a yellowish-grey to a brownish-red.

Bacillus subflavus occurs in rods two to four times as long as broad, often associated in clusters. It forms pale yellow pigment, which in plate cultures shines like mother-of-pearl. The pigment is more apparent in agar-agar cultures.

Bacillus brunneus, a small non-motile bacillus. In characteristic cultures the medium surrounding the growth exhibits a diffuse brown colour.

(b.) NON-CHROMOGENIC.

Bacilli resembling Typhoid Bacilli.—Weichselbaum has described a group of motile bacilli which in their morphological and cultural characters resemble both the typhoid and colon bacillus.

On Plate Cultures the growth is similar to that of the typhoid and colon bacilli.

On Potatoes the growth is sometimes brown, sometimes yellow, and sometimes scarcely visible.

Milk is coagulated.

Grape-Sugar is fermented by some forms, and by others it is not.

The *Nitroso-indol* reaction is sometimes positive, sometimes negative. That this group contains a number of different organisms is

shown by the fact that it is not possible with any one of these species to produce immunity against another. Also in experiments with the blood-serum of animals, rendered immune against one of these species, the power of causing agglutination in the culture of another species was always absent.

2. *Bacilli which liquefy Gelatine.*

(a.) CHROMOGENIC.

Bacillus arborescens.—Slender non-motile bacillus, frequently forming wavy threads; distinguished by the branch-like ramifications and iridescence of the colonies in gelatine plate cultures.

On Potatoes it forms a yellow or orange pigment.

Bacillus fluorescens liquefaciens.—A motile bacillus, very like the *B. pyocyaneus*. It liquefies gelatine very quickly, forms a greenish-yellow pigment, the cultures being fluorescent.

On Glycerine Agar cultures are quite typical, exhibiting an olive-green to olive-brown colour.

Bacillus rubidus.—Medium-sized actively-motile rods, arranged in long threads. A brownish-red pigment is produced in gelatine, agar, and potato cultures.

Bacillus violaceus.—Small, slender, actively-motile bacilli, forming, in agar cultures, spores in the middle of the rods. Cultures on gelatine exhibit in the liquefied portions a bluish violet-coloured bacterial mass. On agar and potato, the formation of the pigment is very abundant, varying from a dark violet to an almost black colour.

Bacillus viscosus.—A bacillus very like *B. fluorescens liquefaciens*, but distinguished by the chocolate-coloured coating it forms on media.

Bacillus janthinus.—Medium-sized motile bacilli; the appearance of a colony growing on a gelatine-plate is comparable to a drop of ink. It forms a violet pigment on all media.

Bacillus helvolus.—Motile rods of varying length, sometimes arranged in short threads, producing a sulphur-yellow coloured pigment. On plates the colonies appear as bright yellow discs lying in a funnel-shaped liquefied cavity. On agar an extensive coating of an intense yellow colour is formed.

Bacillus prodigiosus.—Very small rods (formerly described as the *Micrococcus prodigiosus*, or *Monas prodigiosa*), often arranged in small

chains, possessing very slight motility. The bacillus is found in the air, less often in water ; it is frequently found on bread and potatoes, on meat, and in milk. It grows on all the ordinary media, producing a bright red colour, which is most intense on potatoes. The pigment does not become diffused into the medium.

On gelatine *Plates* the deep colonies are like white points, while the surface colonies are round and red in colour, with irregular borders. The *gelatine* is very quickly liquefied. When cultivated at 37° C. for several generations, the bacillus loses the power to form the red pigment. In the cultures on potatoes it forms *trimethylamin*.

Milk is coagulated. Sugar media are fermented. The bacillus grows also in the absence of oxygen, but no red pigment is produced. It is non-pathogenic, excepting in huge doses.

(b.) NON-CHROMOGENIC.

Bacillus liquefaciens.—One of the most common and widely distributed water bacilli. Actively motile rods, often arranged in chains of four or more. Grows at room temperature, but not at 37° C. Gelatine very quickly liquefied.

On *Plate Cultures* it forms slimy white colonies.

In *Gelatine Stab-Cultures* it forms a funnel-shaped liquefaction. The cultures give off an unpleasant odour. It is a facultative anaërobe.

Bacillus liquidus.—A water bacillus, occurring in the form of short, plump, slightly motile rods, which liquefy gelatine very quickly. In gelatine stab-cultures the liquefied gelatine is covered with a thin membrane, which, when the tube is shaken, sinks to the bottom of the fluid.

Bacillus aquatilis.—A water bacillus, occurring in the form of thin motile rods, which liquefy gelatine slowly (according to some authorities, not at all). In gelatine stab-cultures the growth occurs on the surface of the medium as small yellow colonies, and on potatoes as a scanty yellowish coating.

Bacillus mycoides.—Found in the earth and in certain kinds of forage. Thick, slightly motile bacilli with rounded ends. Spores are formed in the middle of the rods. Grows only in the presence of oxygen. The greyish-white colonies consist of a network of fine twisted threads. Gelatine is liquefied.

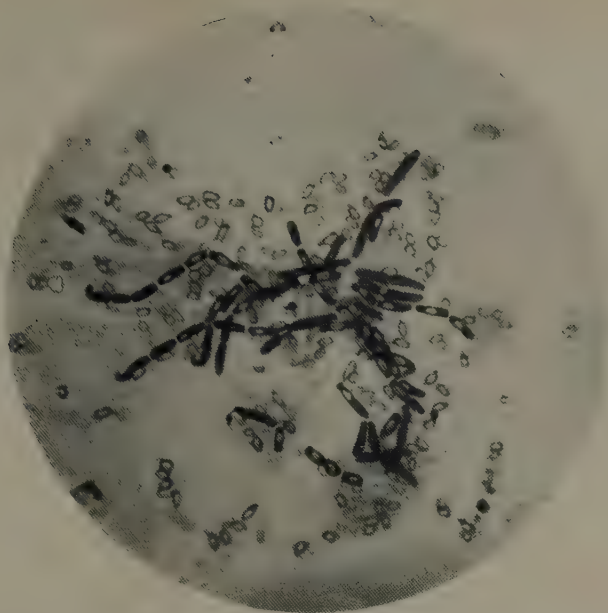


FIG. 106.—*B. subtilis*. Stained with fuchsin, the spores unstained. $\times 1000$.

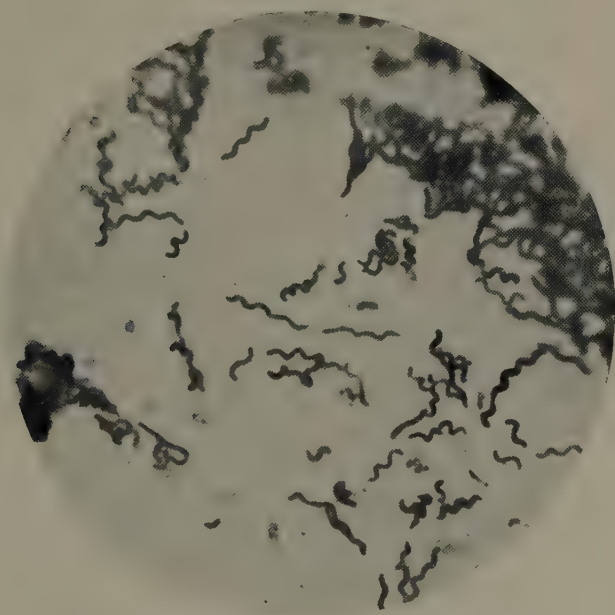


FIG. 107.—*Spirillum rubrum*. Stained with fuchsin. $\times 1000$.

In Stab-Cultures the growth resembles an inverted pine tree.

On Agar the growth resembles the branching of the roots of a tree.

Bacillus mycoides roseus morphologically resembles the *Bacillus anthracis*. It grows best at room temperature. On gelatine plates it forms round, scanty, quickly liquefying, red-coloured colonies. On agar a red-coloured growth. The pigment is only formed in the dark; exposed to the light, the growth is white. The pigment is soluble in water.

Bacillus subtilis (Hay Bacillus).—Large motile rods (see Fig. 106), frequently growing in long straight threads; aërobic; liquefies gelatine quickly. Optimum temperature, 30°; minimum, 10°; maximum, 45° C.

On Plate Cultures bright greyish-white colonies appear, surrounded by a radiating margin.

On Agar the growth presents a shrivelled appearance, and is easily detached. Spores form in the middle of the rods, being somewhat broader, but considerably shorter than the mother cell. The hay bacillus is found in the air, water, dust, fæces, hay, etc. To obtain pure cultures proceed as follows:—Cut some hay into small pieces; place it in an Erlenmeyer flask; cover it with water, and boil for fifteen minutes. By this means all germs are destroyed, except the *resistant spores* of the hay bacillus. These subsequently sprout, and a thin membrane of hay bacilli forms in two to three days on the surface of the hay-infusion.

Bacillus mesentericus (Potato Bacillus).—There are three species of the organism described:—

1. *B. mesentericus vulgatus*.
2. *B.* „ *fuscus*.
3. *B.* „ *ruber*.

The last form communicates a rose colour to the potato on which it is growing. Its spores endure boiling for five to six hours. The spores are very large in comparison to the mother cell. The cultures resemble those of the hay bacillus. *Milk* is coagulated and peptonized.

Bacillus spinosus.—An anaërobic motile bacillus found in garden soil. In gelatine it forms opalescent spherical colonies with bristly outgrowths. Gelatine is liquefied, and gas formed. The stab-culture prior to the occurrence of liquefaction resembles a hairy caterpillar.

The bacillus grows at room-temperature and at 37° C. The spores are formed in the middle of the rods, which become enlarged like a spindle ("clostridium").

II.—MICROCOCCI.

1. *Which do not liquefy Gelatine.*

(a.) CHROMOGENIC.

Micrococcus aurantiacus.—Round or oval cocci; arranged in small clusters. In cultures the colonies are yellow, shiny, circular or elliptical, and do not extend very far over the media. Growth aërobic.

Micrococcus versicolor.—Small cocci, arranged in small groups or in the form of diplococci. They are very frequent in the air. The cultures present an irregular form and yellowish-green colour. On gelatine they exhibit mother-of-pearl-like iridescence. They cause fermentation in grape-sugar media.

(b.) NON-CHROMOGENIC.

Micrococcus candicans.—Round medium-sized cocci, best recognised by their growth in gelatine stab-cultures, in which they form a nail-like growth with a porcelain-white shiny head.

Micrococcus concentricus.—Small cocci, arranged like bunches of grapes (Staphylococci), characterised by the concentric growth of the colonies on gelatine plates and in stab-cultures. The colonies vary in colour from white to a bluish-grey, and are indented on the surface.

Micrococcus rosettaceus.—Medium-sized cocci. The growth is frequently superficial, the colonies having the form of rosettes, with irregular edges of a greyish colour, the central portion varying from dark grey to brown.

Micrococcus aquatilis.—Round light-grey colonies with a mother-of-pearl lustre, the edges appearing indented. Under a low power the colonies resemble a berry in shape.

2. *Micrococci which liquefy Gelatine.*

(a.) CHROMOGENIC.

Micrococcus cremoides.—Small cocci, arranged in bunches, and forming a cream-coloured pigment. At first yellowish-white to

brownish-green granular circular colonies develop on gelatine ; later, the bacterial mass lies in a liquefied depression.

Micrococcus agilis.—Actively motile coccus, in which flagella have been demonstrated. It grows on the different media, forming a rose-coloured pigment. Gelatine is slowly liquefied.

Sarcina lutea (Yellow Sarcina).—Aërobic coccus, arranged in bale-like form. On gelatine plates, round, slightly granular, yellow colonies develop.

In Stab - Cultures it shows a vigorous growth upon the surface. Gelatine is very slowly liquefied, and the citron-yellow bacterial mass sinks to the bottom of the tube, while the upper portion of the medium remains clear.

Besides the yellow Sarcina, there are *white*, *orange*, and *red* Sarcinæ, which are only distinguished from the above-mentioned yellow form by the different colours of the pigment produced. These Sarcinæ are all found in the air.

(b.) NON-CHROMOGENIC.

Micrococcus radiatus.—Small cocci with no typical arrangement. On plate - cultures the colonies possess a radiating border. In stab-cultures the growth exhibits horizontal rays. Gelatine is slowly liquefied.

III.—VIBRIOS AND SPIRILLA.

Vibrio aquatilis (Günther).—This vibrio is easily distinguished from the cholera-vibrio by the character of its growth in cultures. It forms smooth-edged, finely granular, circular colonies. Later, when the gelatine commences to liquefy, the colonies show a faint resemblance to those of the cholera-vibrio. It is further distinguished from the cholera-vibrio by the nitroso-indol reaction being negative, as well as by the absence of pathogenic properties. When first isolated it grew badly in fluid media, but after many generations cultures were obtained in bouillon and peptone water. Günther failed to observe the formation of spiral chains.

Vibrio berolinensis.—Found by Neisser in Berlin conduit-water. On gelatine plates the edges of the colonies are mostly smooth, and exhibit a much more granular appearance than the colonies of the cholera-vibrio. Gelatine is slowly liquefied, and cultures give the nitroso-indol reaction. Guinea-pigs inoculated intraperitoneally, die

with the same symptoms as those following upon the introduction of the cholera germ. Similar vibrios have been isolated by Weibler, Löffler, Fokker, Kiesling, and also from the river Seine.

Vibrio metschnikovi.—This vibrio was first found in an epidemic amongst chickens, then in water from the river Spree. It is somewhat thicker and shorter than the cholera-vibrio, often exhibiting a coccoid appearance. It is actively motile. The cultures resemble those of the cholera-vibrio, but the liquefaction of gelatine is more pronounced, and already, after twenty-four hours, cultures give a well-marked nitroso-indol reaction. In contradistinction to the cholera-vibrio, it is just as pathogenic for pigeons as for guinea-pigs.

Vibrio gindha (Pasquale).—Found in well water at Massauah. Somewhat long, slightly-bent rods, actively motile, and possessing one flagellum. Slightly pathogenic. Nitroso-indol reaction negative.

Vibrio lissabon.—Obtained in a widespread cholera epidemic in Lisbon, in which only one death occurred. On gelatine plates it forms circular, sharply defined, slightly liquefying, whitish-yellow colonies. Nitroso-indol reaction negative.

Vibrio phosphorescens (Dunbar).—Isolated from the river Elbe. Morphologically and in cultures it resembles the cholera-vibrio, but is distinguished by being phosphorescent.

Vibrio massauah.—This vibrio possesses two to four flagella, while the vibrio of Asiatic cholera has usually but one. The nitroso-indol reaction is positive. It is pathogenic for pigeons, guinea-pigs, and rabbits.

Vibrio rugula.—Found in fæces, water, and deposit on the teeth.

Microscopical Appearances.—Slightly bent, finely granular, motile rods (see Fig. 108). Flagella situated at the end of the organism in bundles.

Biological Characters.—Optimum temperature about 37° C.

On Gelatine Plates it forms irregular white colonies, the surface colonies consisting of delicate tufts.

On Agar the surface growth is slight, the development in the condensation water being much stronger.

On Blood-Serum is not liquefied ; growth luxuriant.

Milk is not altered.

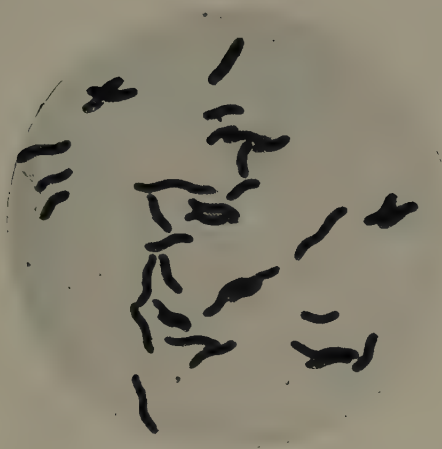


FIG. 108.—*Vibrio rugula*, with flagella. Agar culture. Stained with Orcein solution. $\times 1000$.



FIG. 109.*—*Spirillum undula*, with flagella. $\times 1000$.

* I am indebted to Herr Hänsel, of Berlin, for this specimen.

Indol formation has not been observed.

It is *non-pathogenic*.

Spirillum undula.—Found in putrid fluids, especially in straw infusions. Thick, actively motile spirilla, the flagella arranged in a bundle at one pole. (See Fig. 199).

Biological Characters.—The optimum temperature appears to lie between 22° and 27° C.

On Gelatine Plates it forms sharply defined, granular, greenish-yellow colonies, while the medium appears to be slightly liquefied.

In Gelatine Stab-Cultures, in the upper portion of the puncture a veil-like clouding of the medium occurs, while round the opening of the puncture a whitish growth with ragged tufted edges develops.

On Agar the water of condensation is clouded, but no film is formed. Kutscher distinguishes two forms—*Sp. undula majus*, and *Sp. undula minus*.

BACILLUS ENTERITIDIS SPOROGENES (KLEIN).*

This organism was isolated by Klein in 1895, during an outbreak of diarrhoea amongst the patients of St Bartholomew's Hospital. It was also found in the alvine discharges in four out of ten cases of fatal infantile diarrhoea, and in six out of eight cases of cholera nostras examined. It has also been isolated from milk, manure, and sewage.

Microscopical Appearances.—When obtained from a typical milk culture or from the exudation of an animal dead of the disease produced by it, the organism consists of rods 1.6 μ to 4.8 μ long, and 0.8 μ broad; some of the rods are longer than others, and many form short chains, and those containing spores are always thicker than the non-sporing forms.

Spore Formation present. The spores appear at first as minute glistening bodies, generally situated near one end of the rod, and gradually increasing in size, become ovoid, and measure 1.6 μ in length and from 1.0 to 1.2 μ in breadth. On blood-serum media at 37° C., spore formation commences in two to three days, and in seven to ten days the whole culture is one mass of free spores.

Motility.—Some of the rods exhibit motility, others do not. The flagella are long, sometimes in bundles situated close to one or both of the rounded ends of the rod.

* Klein, *Micro-organisms and Disease*, 1896, pp. 392-397.

Staining Reactions.—The bacillus stains by the Gram method and also with the ordinary anilin stains; the spores can also be stained by the usual methods.

Biological Characters.—This organism can only be cultivated under anaërobic conditions. Buchner's method (see p. 82) is specially applicable for this purpose.

Pathogenesis.—Guinea-pigs injected intraperitoneally with bacilli from young cultures die in six to eight hours. Spores alone, or cultures five to seven days old, in which spore-formation is nearly completed, are not as virulent as young cultures when injected subcutaneously into guinea-pigs. Mice are also susceptible. Five minims of a subcutaneous exudate from a previously-infected animal produced a fatal infection in twenty to twenty-four hours.

BACTERIA FOUND IN MILK.

Bacteria are always present in milk, unless it is drawn from the udder under aseptic precautions. Milk may be contaminated in various ways: from the gland direct, *i.e.*, *B. tuberculosis*, etc.; through the soiled hands of the milker, dirty vessels, hay, dust, etc., contaminated well water (*B. typhi*), and by the water added by the thrifty dairyman. Milk is an excellent medium for the development of many forms of bacteria, and under favourable conditions of temperature the germs quickly multiply.

Pathogenic bacteria may also contaminate milk. The organism most frequently found being the *B. tuberculosis*. Pyogenic cocci have also been found. From contamination with matter derived from diseased persons, milk may be the means of carrying and spreading typhoid fever, diphtheria, and scarlet fever. Such organisms are, however, easily destroyed. The *B. tuberculosis* is killed by *Pasteurising* the milk for thirty minutes at 70° C., or by boiling it for ten minutes. Such sterilization is but partial, as it does not destroy the resistant spores of certain non-pathogenic bacteria. (For methods of examining bacteria in milk, see p. 25.)

A.—MICROCOCCI.

Micrococcus acidi lactici.—Found in fresh milk, occurring either as single large cocci or diplococci. Aërobic. Forms small, yellowish,

non-liquefying colonies on gelatine plates. Litmus-milk is first coloured red, then coagulated, and finally becomes decolorized.

Sphærococcus acidii lactici.—Found in fresh milk; small cocci, occurring as diplococci or arranged in clusters.

Biological Characters.—Facultative anaërobe.

On Gelatine Plates.—Round, white, non-liquefying colonies.

Milk is coloured red and coagulated with the formation of acid.

Streptococcus acidii lactici.—Found in curdled milk; small cocci, arranged in chains.

Biological Characters.—*On Gelatine Plates*, round, white, non-liquefying colonies.

In Gelatine Stab-Cultures the growth is entirely confined to the stab.

Milk is coagulated with the formation of acid.

Micrococcus acidii lactis liquefaciens.—Found in butter; oval cocci, occurring in diplococci or tetrads.

Biological Characters.—Facultative anaërobe; optimum temperature, 21° C. Liquefies gelatine.

On Gelatine Plates.—Small, round, white colonies.

In Gelatine Stab-Cultures.—Funnel-shaped liquefaction, with a film on the surface.

Milk is coagulated with the formation of acid. The casein is not peptonised. After one to two weeks it acquires a musty odour.

Streptococcus hollandicus.—Found in the ropy whey used in making Edam cheese. Occurs in pairs, and frequently in long chains. When sterile milk is inoculated with this organism, it becomes sour and ropy in twelve to fifteen hours at 25° C.

B.—BACILLI.

Bacillus anaërobius, II., III., IV. (Flügge).—Three different species of bacilli found several times by Flügge in milk that was cooked one and a half hours. Species III. and IV. form spores. They are all anaërobic, rapidly liquefy gelatine, and form gas quickly in sugar media.

In Milk, No. II. causes coagulation without gas formation.

No. III. has no effect.

No. IV. coagulates milk with gas formation.

Pathogenesis.—Nos. III. and IV. are poisonous to animals, while No. II. is not.

Bacillus cyanogenus.—This organism, the cause of blue-coloured milk, was first cultivated by Hueppe on gelatine plates.

Microscopical Appearances.—Bacilli of 0.3 to 0.5 μ broad by 1 to 4 μ long.

Motility.—Motile, the flagella being arranged in a bundle at one end.

Spore Formation.—Absent. The "spores" described by Hueppe are considered by Heim to be due to degeneration.

Staining Reactions.—Stains with the ordinary dyes, but not by the Gram method.

Biological Characters.—Grows best at room temperature; almost no growth takes place at 37°. It does not liquefy gelatine, and is non-pathogenic.

On Gelatine Plates.—Greyish-white, granular colonies, with scalloped edges.

In Stab-Cultures the deep growth is very limited.

On Agar.—Greyish-blue growth.

On Potatoes.—A yellowish shiny coating. Two pigments are formed, a blue and a fluorescent colour. In gelatine cultures the fluorescence appears first, and later the blue-black pigment. The latter develops more luxuriantly on agar.

In Milk the blue pigment is only formed in the presence of an acid. In unsterilized milk blue spots appear at first on the cream; finally the whole surface is coloured a sky-blue.

In Sterile Milk a grey colour develops, which only turns blue on the addition of an acid. If grape-sugar is added to sterile milk the latter is coloured blue by the bacillus, acid being formed from the sugar.

This bacillus was supposed to cause the blue colour in cheese, but such a supposition is untenable in view of the inoculation experiments conducted by Adametz and Beyerinck.

Bacillus acidi lactici (Hueppe).—Found in sour milk. This organism is without doubt identical with the *Bacterium lactis* discovered by Lister in 1877. Lister also discovered that lactic acid bacteria, although frequently found in dairies, are very seldom found in the open air.

Microscopical Appearances.—Non-motile rods 1.0 to 1.7 μ long and 0.3 to 0.4 μ broad, mostly in pairs, but sometimes arranged in small chains.

Spore-Formation absent.

Biological Characters.—Facultative anaërobe; optimum temperature 37° C.

On Gelatine Plates.—Forms flat, diffuse, superficial colonies with irregular borders, resembling those of *B. coli*.

In Gelatine Stab-Cultures.—A nail-shaped growth.

On Agar.—A yellowish-white coating.

On Potatoes.—A yellowish-brown coating.

In Milk it forms an acid which precipitates the casein.

The above is not the only organism that changes milk-sugar into lactic acid, but it is probably the common cause of the spontaneous souring of milk.

Bacillus lactis acidii (Leichmann).—This bacillus was found by Leichmann in milk, which underwent spontaneous lactic acid fermentation at 50° C. When cultivated in sterile milk it forms lactic acid, which turns polarised light to the left.

Bacillus lacticus (Günther and Thierfelder).—Found in sour milk.

Non-motile bacilli, 0.5 to 0.6 by 1.0 μ , usually arranged in pairs or small chains. It does not produce spores. It stains by the Gram method.

Biological Characters.—Grows best at 28° C., and on media containing sugar.

Gelatine is not liquefied. The developing colonies remain very small.

On Potatoes the development is very scanty.

In Bouillon.—A turbid growth occurs, which does not cause any change in the reaction.

In Grape and Milk Sugar Bouillon the growth is luxuriant, the media becoming strongly acid without the production of gas.

To obtain pure cultures of the organism from sour milk proceed as follows:—

1. Melt three tubes of gelatine in the water-bath at 30° C.
2. Put some CaCO_3 in a clean tube, add a little water, and sterilize at 100° C.
3. Inoculate the first tube with three platinum loops of milk, and

dilute in the usual manner, using five platinum loops of milk. Mix the milk and gelatine thoroughly.

4. Put a few drops of the solution of CaCO_3 in three sterile Petri-dishes, pour the inoculated gelatine on the CaCO_3 solution, and see that they are thoroughly mixed.

After development each acid-forming colony will be found surrounded by a transparent zone.

Bacillus lactis (Flügge).—Found in bitter milk. Flügge isolated eleven species, which all belong to the group of hay bacilli. They possess the power of peptonising the casein of the milk, whereby it acquires a bitter taste. A few of the varieties produce toxic substances which, when given to young dogs *per os*, cause diarrhœa, muscular weakness, and a fall of temperature. They form very resistant spores, which are not destroyed by several hours' boiling. It is on account of these spores that sterilization of milk is so difficult. If improperly sterilized milk is placed at a suitably high temperature, the spores germinate, and the bacilli produces toxins. Some authors consider that the toxins cause summer diarrhœa in children.

The morphological characters of these eleven species are similar to those of *B. subtilis*. They are all motile rods of various lengths, and all liquefy gelatine.

Bacillus lactis inocuus.—Found in milk and in the fæces of infants. Short, non-motile rods forming capsules in the body.

Biological Characters.—Aërobic. *Pathogenic* only in very large doses.

On Gelatine Plates white, round, non-liquefying colonies develop.

On Potatoes a brownish coating.

Milk is not changed. No gas is formed in grape-sugar agar. Indol is not formed.

Bacillus lactis albus (Löffler).—Found in butter-milk. Very long, motile, spore-forming bacilli, arranged sometimes in threads.

Biological Characters.—Gelatine is liquefied.

On Agar a thick whitish coating develops. *On Potatoes* dry white colonies. *Milk* is coagulated, and the casein peptonised.

Bacillus lactis (Bleisch).—Found in butter-milk; large motile bacilli, forming spores. The spores are very resistant, and are only killed after being boiled for six hours. Facultative anaërobe. *Gelatine* is liquefied. *On Agar* and *Potatoes* a light-grey coating.

Bacillus lactis erythrogenes (Hueppe).—Found in red-coloured milk. Short, non-motile rods 0.3 to $0.5\ \mu$ by 1 to $1.4\ \mu$.

Spore Formation absent.

Biological Characters.—On *Gelatine Plates*, round, yellow, slowly liquefying colonies; the gelatine surrounding the colonies is coloured red. Non-pathogenic.

In Gelatine Stab-Cultures the development is very slow. When kept in a dark place, the medium is coloured red.

On Agar and Potatoes a yellowish coating develops, the surrounding medium being coloured a faint red.

Milk is fermented and peptonised, bad-smelling gases being formed. The milk acquires at first a dirty red, then brownish-red, and finally a blood-red colour.

Bacillus lactis pituitosi (Löffler).—Obtained by Löffler from milk.

Microscopical Appearances.—Somewhat thick, slightly bent rods, that soon break up into coccoid segments.

Biological Characters.—In gelatine it forms white colonies which, by transmitted light, appear brown-coloured, usually sharply outlined, but sometimes indented.

On Agar, dirty whitish colonies develop.

On Potatoes, a greyish-white, pearly, somewhat dry coating.

Milk becomes slightly acid and slimy, giving off a quite specific odour. Whether this ropy mass is formed from the milk-sugar or from the casein has not been determined.

Bacillus limbatus acidi lactici.—Found in milk.

Microscopical Appearances.—Short, non-motile rods, mostly in pairs, possessing a capsule but not forming spores.

Biological Characters.—On *Gelatine Plates*, round, white, non-liquefying colonies.

In Gelatine Stab-Cultures the growth is mostly on the surface.

Milk is coagulated, acid formed.

Bacterium acidi lactici (Grotenfeld).—Found in sour milk. Small, non-motile bacteria 0.3 to $0.4\ \mu$ by 1 to $1.4\ \mu$.

Biological Characters.—Facultative anaërobe; optimum temperature, 37°C .

On Gelatine Plates, round, porcelain-white colonies.

In Gelatine Stab-Cultures a nail-like growth.

On Potatoes a greyish covering.

Bacteria Causing Ropiness in Milk.

Bacillus gumosus.—Found in slimy milk ; large, slightly motile, sporogenic bacilli.

Biological Characters.—Gelatine is liquefied slowly.

On Agar and Potatoes, a puckered white coating. Cane-sugar is fermented, and when alcohol is added to the solution gum is formed.

Bacillus viscosus lactis (Adametz).—Found in water.

Microscopical Appearances.—Non-motile capsulated bacilli, 1.1 to 1.3 μ by 1.2 to 1.7 μ ; sometimes occurring in threads.

Biological Characters.—In *Gelatine* the deep colonies are small, while the surface colonies exhibit an extensive development in the form of slimy drops with serrated edges.

On Agar, a dirty-white, ropy, slimy coating develops.

Milk at ordinary temperature becomes ropy in five to ten days, and finally transparent, from disintegration of the milk globules.

Bacterium acidi lactici (Peters).—Found in sour dough. Short motile rods, 0.4 to 1.5 μ .

Biological Characters.—On *Gelatine Plates* round colonies of a light red colour, with concentric stratification. In solutions of sugar, to which some yeast is added, this organism produces great quantities of lactic acid.

BACTERIA CAUSING ACETIC ACID FERMENTATION.

Bacillus aceticus (Hansen).—The "*Mycoderma aceti*" was considered by Pasteur to be the cause of the acetic acid fermentation in wine and beer. (Hansen recognises two species, *Bacillus aceticus* and *pasteurianus*.)

Microscopical Appearances.—Short, non-motile, non-sporogenic bacilli, forming threads and frequently involution forms.

Staining Reactions.—Stains by the Gram method.

Biological Characters.—Aërobic ; optimum temperature, 30° to 40° C.

On Solid Media it grows on the surface, forming porcelain-white cup-like colonies, especially on *Beer Gelatine* (prepared by adding 5 per cent. gelatine to beer). In *Liquid Media* a membrane forms on the surface, the underlying liquid being slightly clouded.

Specific Actions.—It oxidizes alcohol, forming acetic acid, and splits up acetic acid into CO_2 and H_2O . It is distinguished from other members of the group by staining yellow when treated with a solution of iodine.

Bacillus pasteurianus (Hansen).—This bacillus is found in beer and wine, but does not occur as frequently as the previous organism, from which it is distinguished by giving a blue reaction with solution of iodine.

Bacillus aceticus petersii.—Found in old, sour dough; resembles the *Bacillus aceticus* of Hansen. Forms threads; is strongly aërobic; on gelatine slimy colonies develop.

BACTERIA CAUSING BUTYRIC ACID FERMENTATION.

Bacillus butyricus (Botkin).—Found in water, milk, and manured earth.

Microscopical Appearances.—Long, motile bacilli, forming threads.

Spores formed in the middle of the rods. The spores are very resistant, and are not killed during the process of sterilizing milk. As they do not germinate at temperatures beneath 18°C ., milk sterilized for children should be kept at a lower temperature before use.

Staining Reactions.—Stains by the Gram method.

Biological Characters.—Anaërobic.

On Gelatine Plates.—Round or oval liquefying colonies, developing an odourless gas.

In Milk a layer of serum forms at the bottom, out of which the gas rises; the milk is coagulated. The coagulated albumen rises to the surface and is peptonised, so that eventually only the fat swims on the surface. Butyric and allied fatty acids are formed. Involution forms occur in media containing sugar.

Bacillus butyricus (Prazmowsky).—Found in putrid vegetable infusions.

Microscopical Appearances.—Actively motile bacilli, about 1μ broad, of varying length, forming threads.

Spores formed in the middle of the rod, causing spindle-formed swelling of the bacillus. The spores are 1μ broad and 2 to 2.5μ long. When the spore germinates the bacillus issues at one end,

the spore membrane remaining attached like a cap to the young bacillus.

Staining Reactions.—With a watery solution of iodine the bacilli, when cultivated on media containing starch, are stained blue, whence it has been also called *Bacillus amylobacter*. It has also been described under the name of *Clostridium butyricum*.

Biological Characters.—Anaërobic; in solutions of starch, dextrin, sugar, and lactic acid salts, a great quantity of butyric acid is formed, accompanied by the development of H and CO₂.

***Bacillus butyricus* (Hueppe).**

Microscopical Appearances.—Large rods, frequently occurring in pairs.

Spores formed in the middle of the rods.

Biological Characters.—Grows in the presence of oxygen on ordinary media at both room temperature and at 37° C.

Gelatine is quickly liquefied.

On slanted Agar, a moist yellowish coating.

In Milk it develops best at 37° C., and causes coagulation similar to that produced by rennet, the reaction of the milk remaining unchanged. The casein is subsequently dissolved and changed into peptone and other products, ammonia being produced. At the same time the milk acquires a bitter taste. This bacillus forms butyric acid out of lactic acid salts.

BACTERIA CAUSING SPECIFIC CHANGES IN BEER, WINE, AND SUGAR.

***Bacillus viscosus cerevisiæ* (Van Laer).**—Found in ropy beer, yeast, in the air, and on slimy bread.

Microscopical Appearances.—Rods 0.8 to 1.6 μ by 2 to 4 μ , seldom occurring in chains; said to form terminal spores.

Biological Characters.—In *Gelatine Stab-Cultures* the growth is uniform along the track of the needle; diffuse white on the surface. The colonies are sharply circumscribed, and when examined under a low power appear brown coloured; old colonies are serrated.

Beerwort at 27° C. is rendered ropy in twenty-four hours, a large amount of CO₂ being formed; later the surface is studded with yellowish, ropy, slimy colonies.

Milk, Solutions of Peptone, and Cane-Sugar also become ropy, gas being formed.

On Potatoes, white, warty, viscid colonies develop, giving off a smell like decayed fish. (Van Laer also describes another similar organism, which is distinguished from the above by only producing slight fermentation and ropiness.) The injurious influence of this organism is only manifest when it obtains access to the wort before the primary fermentation. When added afterwards no injurious action is observed. The ropiness in the beer is due to two mucilaginous substances produced by this organism: one contains nitrogen and is insoluble; the other contains no nitrogen and is soluble in water.

Bacillus viscosus sacchari (Kramer).—Found in slimy solutions of sugar.

Microscopical Appearances.—Small, non-motile rods, forming threads but no spores.

Biological Characters.—Optimum temperature, 22° C.

Stub-Cultures in cane-sugar gelatine liquefy quickly, an adherent sediment being formed.

Bacillus viscosus vini.—Found in slimy wine.

Microscopical Appearances.—Non-motile bacteria of varying lengths, forming threads.

Biological Characters.—Anaërobic; optimum temperature, 18° C. Develops only on wine and glucose solutions. Wine is fermented in one to two months, a thick slime being formed.

Leuconostoc mesenteroides (Cienkowski).—*Ger.* Froschlaichpilz; Pilz der Dextrangährung.

Found in beetroot juice and molasses of sugar factories, where it develops in large gelatinous masses, resembling *frog spawn*. It is also found on raw or cooked carrots and sugar beets.

Microscopical Appearances.—It forms chains of spherical or oval cocci from 1.8 to 2 μ in diameter, enclosed within a thick, tough, membranous envelope. Owing to the anastomoses of numerous chains they appear as large, compact, gelatinous, zoöglæic masses.

Staining Reactions.—The cover-glass specimen is first stained with dahlia violet, which stains the cocci, and then immersed in an aqueous solution of rosolic acid, which stains the gelatinous envelope a rose-red colour.

Biological Characters.—It is a facultative anaërobe; optimum temperature, 30° to 37° C. It is very difficult to obtain in pure cultures, owing to the gelatinous mass being contaminated with various fungi. To overcome this difficulty, heat the cultures continuously for fifteen minutes at 75° C., in order to destroy the fungi. The gelatinous envelopes ($C_{12}H_{10}O_{10}$) only develop in media containing cane or grape-sugar.

On ordinary Gelatine the growth exhibits no special characteristics. *On Gelatine* containing grape-sugar it is very characteristic; consisting after ten to fourteen days of a whitish, confluent mass, with slimy, hyaline, gelatinous lumps on the surface. During the first eight days the growth exhibits a dry elastic consistence, but during the next few weeks it becomes softer, moister, finally forming a soft pulp. Individual colonies present a warty appearance, though sometimes they spread on the surface in the form of a puckered film.

As already mentioned, the gelatinous substance is only formed in material containing grape or cane-sugar. Other carbohydrates tested by Leisenberg and Zopf were found unsuitable. The organism produces *invertin*, which splits up the cane-sugar. It ferments lactose, maltose, and dextrin, forming lactic acid with a faint evolution of gas. The addition of 3 to 5 per cent. of calcium chloride to the nutrient medium favours the production of mucus and the fermentative activity of the organism. Fermentation is more active when oxygen is excluded.

Leuconostoc indicum.—This organism is the cause of considerable damage to the Java sugar industry. According to Leisenberg and Zopf, the only difference between this organism and *Leuconostoc mesenteroides* is a slight difference in the optimum temperature at which it develops.

Ascoccoccus billrothii.—Found by Billroth in putrefying meat-infusion.

Microscopical Appearances.—Small cocci, arranged in peculiar small spherical or oval colonies, which form a creamy layer upon the surface of liquid media. The colonies consist of a jelly-like, extremely resistant envelope, from 10 to 15 μ thick. One or more masses of cocci, from 20 to 70 μ or more in diameter, are situated within the envelope. The cocci are closely arranged, and united by firm but scanty intercellular substance.

Biological Characters.—Aërobic. Grows at ordinary room temperature. Produces a strongly alkaline reaction (Ammonia) in culture media.

According to Cohn, it produces a greenish-white slimy mass upon slices of beetroot, and a slimy fermentation in the juice of sugar beets.

THE PHOSPHORESCENT BACTERIA.

Bacterium phosphorescens (Fischer).—Found on dead meat and fish. Occurs as short, non-motile rods, sometimes in zoöglææ. It forms no spores, and stains by the Gram method.

The bacillus is a facultative anaërobe, does not liquefy gelatine, and only grows on media containing chloride of sodium. In gelatine stab-cultures the growth is usually on the surface. The blue-green phosphorescence is best seen on cultures on dead fish, meat, and sea-water.

Bacillus argenteo-phosphorescens (Katz).—Found by Katz in Australia in sea-water, and on dead marine animals. There are three slightly different species.

They occur in motile rods 0.6 to 0.8μ by 2.5μ in size, and stain by the Gram method.

Culture media are not liquefied. Yellowish colonies develop, exhibiting a silvery-white phosphorescence.

Bacillus phosphorescens (Giardi).—Found on living and dead crustaceans. It is pathogenic for crustacea, especially for certain species, which it kills in six to nine days, the whole body of the animal being covered with greenish phosphorescence. When cultivated on ordinary media the bacilli lose their virulence, but regain it when cultivated on fish media. Morphologically and in the cultures the appearance is very similar to *B. phosphorescens*, only smaller and more coccus-like.

Bacillus phosphorescens indicus (Fischer).—Cultivated by Fischer from phosphorescent sea-water in the West Indies.

Microscopical Appearances.—Motile rods, twice as long as broad (0.6 to 0.8μ by 2μ), often arranged in short threads.

Staining Reactions.—Stains with ordinary dyes, but not by the Gram method.

Spore Formation not observed.

Biological Characters.—Grows under aërobic conditions at medium temperatures.

On Gelatine Plates it forms round, bluish-green, slowly liquefying colonies, which later become granular and of a brownish colour.

In Gelatine Stab-Cultures funnel-shaped liquefaction occurs; the growth is limited in the deeper portion of the medium.

On Agar and *on Potatoes*, cooked in sea-water, a dirty whitish coating develops. Blood-serum is liquefied. The blue phosphorescence is very well marked in cultures on dead sea animals, in sea-water, and meat. The organism is non-pathogenic.

Bacillus phosphorescens indigenus (Fischer).—Found by Fischer in Kiel harbour. It is similar to *B. phosphorescens indicus*, only gelatine is liquefied more slowly, while blood-serum is not liquefied. It grows at lower temperatures, and causes no phosphorescence on meat.

THERMOPHILIC BACTERIA.

Miquel found a bacillus in the Seine in 1891, which possessed the faculty of growing at a temperature of 69° to 70° C. In 1887, Koch and Globig found bacteria in surface earth, which grew between 50° and 70° C. M'Fadyean, and also Rabinowitsch, have contributed to our knowledge of this group of bacteria.

The thermophilic bacteria are mostly bacilli that are facultative anaërobes and non-pathogenic. Most of the forms produce spores which exhibit great resistance. The known species grow at a temperature between 56° and 70° C.

BACILLUS CAPSULATUS (PFEIFFER).

Pfeiffer isolated this bacillus from the purulent peritoneal exudate and blood of a dead guinea-pig.

Microscopical Appearances.—A plump bacillus with rounded ends, possessing a well-defined ovoid capsule.

Motility.—Non-motile.

Spore Formation has not been observed.

Staining Reactions.—The reaction with the Gram method is

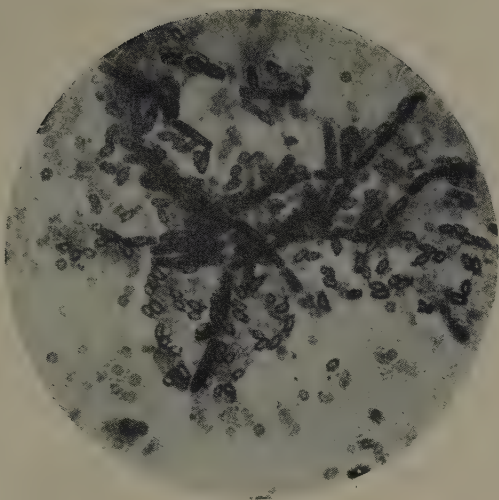


FIG. 110.—*B. megaterium* and spores from a culture. Fuchsin. $\times 1000$.

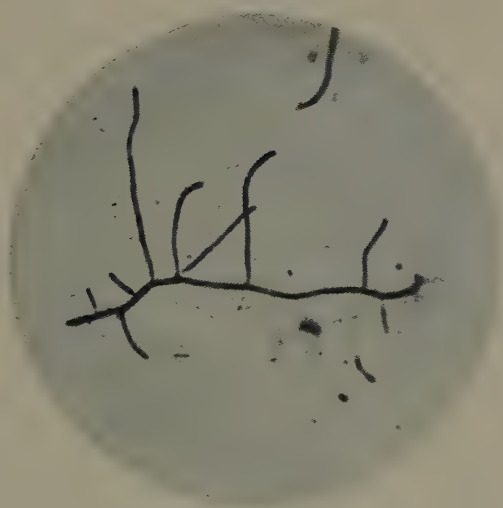


FIG. 111.—*Cladotrix* found in water. Cover-glass specimen from gelatine culture. Fuchsin. $\times 1000$.

negative ; the capsules are easily demonstrated when stained by John's method. (See p. 28.)

Biological Characters.—Facultative anaërobe growing on the ordinary nutrient media, better at 37° C. than at ordinary room temperature.

In Gelatine Stab-Cultures a slimy, white, nail-shaped growth develops, and an inodorous gas is formed ; the gelatine is not liquefied.

On Slanted Agar it forms a thick, moist, white, viscid coating.

On Potatoes, a yellowish-white viscid covering.

Pathogenesis.—White and house mice inoculated subcutaneously die in two to three days. The spleen of the dead animals is found greatly enlarged, and the bacilli, with well-defined capsules, are present in the blood and organs. Guinea-pigs, pigeons, and rabbits are also susceptible, guinea-pigs and pigeons only by intraperitoneal infection, and rabbits only when large quantities of the culture are introduced intravenously. The bodies of the animals very quickly undergo putrefactive changes. The blood and tissue juices are viscid.

BACILLUS MEGATERIUM (DE BARY).

Found in earth, air, and on the leaves of cooked cabbages.

Microscopical Appearances.—Very long ; sometimes 10 μ long and 2.5 μ thick ; slightly bent bacilli, with round ends. Involution-forms often present.

Motility.—Slightly motile, and possessing four to eight flagella, arranged on the sides.

Spore nearly as long as the cells in which they are formed. (See Fig. 110.)

Biological Characters.—Aërobic ; optimum temperature, 20° C.

On Gelatine Plates, kidney or sickle-shaped granular colonies are formed, which liquefy the medium slowly.

On Agar, a whitish coating.

On Potatoes, a thick, greyish-yellow coating.

On all the media it forms a slimy mass ; and, according to Günther, should be grouped with the capsule bacteria.

BACTERIUM ZOPFII.

Found by Kurth (1883) in the intestinal contents of a chicken. It

has also been found in water and fæces ; Günther (1897) found it in sausages.

Microscopical Appearances.—Short, plump bacilli, 2 to 5 μ long, and 0.75 to 1 μ broad, forming long, short-jointed chains.

Motility.—Motile.

Staining Reactions.—Stains with the ordinary methods, and also by the Gram method.

Biological Characters.—Aërobic. The growth is not so luxuriant at 37° C. as at lower temperatures.

On Gelatine Plates.—Examined with a low power, the colonies are round, and consist of long, coiled-up threads.

In Gelatine Stab-Cultures the growth takes place only on the surface, and consists of finely-arranged radiating threads.

On Agar, at 37° C., a thin, greyish coating develops.

In Bouillon, at 37° C., the growth is hardly visible.

Milk is not altered.

The reaction of Grape and Milk-Sugar Media is not changed. The organism does not form indol, and it is non-pathogenic.

CLADOTHRICES.

These organisms are found in water, and consist of colourless thread-like bacteria, which do not contain sulphur grains. The principal characteristic of this group is the *false* branching of the threads. Three species of *Cladothrix* are well known.

Cladothrix dichotoma (F. Cohn).—Found both in standing and running waters, which are more or less rich in organic substances. It is frequently associated with *Beggiatoa*. It occurs in bushy tufts, 1 to 3 mm. high, which are attached to a substratum, or float freely in the water. From the apex of the threads elements (*gonidia*) become detached and swim about freely for a time, then become stationary, and develop into fresh threads. The threads possess a distinct sheath. In solutions of extract of meat a thin film forms, which extends over the surface of the media and up the walls of the tube. In meat extract gelatine, ramifying colonies, causing very slight liquefaction of the medium, develop. (See Fig. 111 of *Cladothrix*, cultivated from drinking water.)

Cladothrix intricata (Russell).—Found in ooze from the bottom of the Gulf of Naples. It is always free, and develops no sheath. It forms an interlaced mass of threads, certain threads showing pseudo-ramification. The fresh threads appear homogeneous, and when stained the bacillus-like elements of which they are formed become distinctly visible. When freed these elements become actively motile, and develop spores which are not thicker than the threads. It is easily cultivated artificially,

On Gelatine Plates, mould-like, rapidly liquefying colonies develop, which under a low power appear to consist of a tangled mass of threads.

In Gelatine Stab-Cultures, radiating processes are given off from the growth.

On Agar, a whitish coating develops, the offshoots of which penetrate the medium.

On Potatoes, a whitish coating.

In Bouillon, a gelatinous sediment is formed.

Cladothrix ochracea (Winogradsky).—Only found in water containing iron salts. Very like the *C. dichotoma*. By oxidation an oxyhydrate of iron is formed, which is deposited in the sheath of the organism. Cultures can be obtained by the addition of iron salts to media.

Orange-pink Cladothrix (Houston).—This organism is mentioned by Houston* as being found in soil. It exhibits a fairly rapid growth in gelatine media, which are liquefied. The orange-pink colour does not diffuse into the medium, being entirely confined to the organism itself.

BEGGIATOIA.

Found in water containing sulphuretted hydrogen. Three species (*B. alba*, *B. roseo-persicina*, and *B. mirabilis*) have been described.

Microscopical Appearances.—Threads without any distinct cell membrane, enclosing in their interior dark grains of sulphur, formed by the oxidation of sulphuretted hydrogen. If a microscopical specimen is prepared and the *sulphur granules* dissolved by adding alcohol or bisulphide of carbon, a distinct system of transverse septa will be visible in the threads.

* Local Government Reports, 1897-98.

Biological Characters.—It can be cultivated in water containing sulphuretted hydrogen, but it exhibits slow growth. Actively motile; flagella have, however, not yet been demonstrated. Should the supply of sulphuretted hydrogen be interrupted, then the small grains of sulphur in the threads are gradually changed into sulphuric acid, and the threads appear completely homogeneous. In time degenerative changes occur, vacuoles being formed. The method by which the *Beggiatoa* multiply is not absolutely known.

BACTERIA FOUND IN LEGUMINOUS NODULES.

The earliest description of the leguminous nodules is by Malpighi, in his book published in 1687, in which he referred to them as galls, *i.e.*, diseased excrescences. In 1853, Treviranus considered the nodules normal growths. In 1866, Woronin discovered that there were numerous entirely closed cells filled with living bacteria within these nodules. In 1879, Frank showed that nodule-formation did not occur when the plants were grown in sterilized soil, thus proving that the co-operation of soil bacteria was a necessary factor. The true nature of these nodule-bacteria was determined by Beyerinck in 1888, who isolated them from the nodules and cultivated them on artificial media. These bacteria are now looked upon as the generators of the nodules, by means of which the *Leguminosæ* are enabled to absorb nitrogen from the air and elaborate it into nitrogenous compounds, albumen, etc. The bacteria are situated in the cells of the inner layer of the nodule, known as the *bacteroidal* tissue, and under the influence of the surrounding protoplasm are modified into involution-forms termed *Bacteroids*. (See Figs. 112 and 113).

For further information on this subject, the reader is referred to an interesting paper on "The Bacteria of Soil, with special Reference to Soil Inoculation," by R. Stewart Macdougall, in the *Transactions of the Edinburgh Botanical Society*, July 1897.

Bacillus radicicola (Beyerinck).—Found in the young root-nodules of leguminous plants.

Microscopical Appearances—In cultures it occurs in the shape of large rods 1 to 4 μ long, and in small clusters of "*rovers*" 0.18 to 0.9 μ . The large rods frequently exhibit knotted, irregular, fork-shaped or three-armed processes. The bacteria in the nodules exhibit

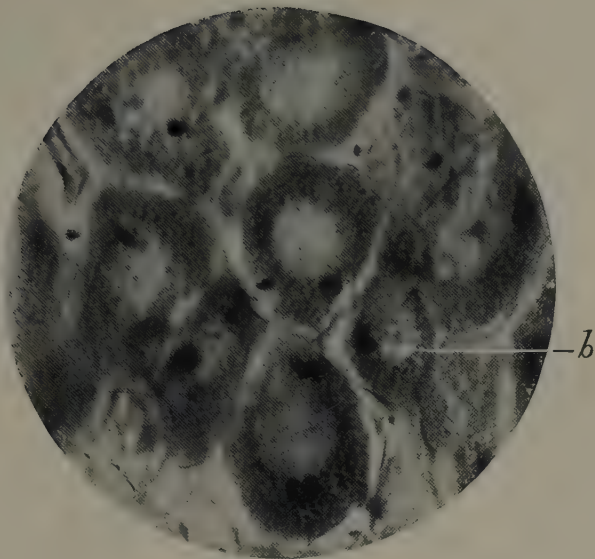


FIG. 112.—Nodule on the root of *Trifolium pratense*, showing cells; and (b) bacteroidal tissue. Stained with methylene-blue. $\times 350$.

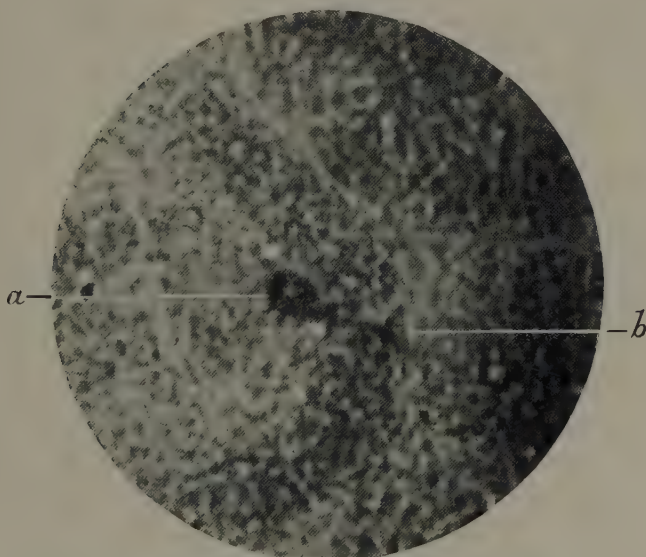


FIG. 113.—Nodule on the root of *Trifolium pratense*, showing cell containing bacteroids (a); and the infection-thread (b). $\times 1000$.

a similar morphology. The *rovers* belong to the smallest of known bacteria, and can pass through the pores of a Chamberland filter.

Motility.—Motile, especially the *rovers*, which sometimes escape from the parent colonies, and found a daughter colony at a distance in the gelatine.

Spore Formation not observed.

Staining Reactions.—Sections of the nodules are best stained in a solution of equal parts of fuchsin and methyl-violet in 1 per cent. acetic acid. The protoplasmic contents and membrane of the nodule-cells are coloured *blue*, the bacteria of the infection-threads *red*, whilst the membrane of the latter remains uncoloured.

Biological Characters.—These organisms grow very slowly under aërobic conditions on ordinary gelatine. The best medium is a decoction of leaves or stalks of Papilionaceæ, with the addition of 7 per cent. gelatine, $\frac{1}{4}$ per cent. asparagin, and $\frac{1}{2}$ per cent. cane-sugar. The colonies are semi-circular, whitish, or slightly clouded. The larger colonies are watery, the smaller ones are solid and cohesive. According to Beyerinck, the various species of Leguminosæ exhibit differences in the form of the colonies and of the bacteria. Kruse considers that the bacilli are probably but varieties of one species.

Rhizobium leguminosarum (Franck).—Franck obtained cultures of this organism in gelatine drop-cultures and on plates. The bacteria occur in the clusters; they are 0.9 to 1.3 μ in length, oval or rod-shaped, and form zoöglææ. Gelatine is liquefied slowly.

Bacillus tuberigenus (Von Gonnermann).—Found in the root-nodules of the lupine, bean, etc. Von Gonnermann, with a specially prepared lupine-peptone-gelatine, isolated seven bacilli, which he named *B. tuberigenus*, Nos. 1 to 7. When sterilized earth was inoculated with Nos. 3 and 5 the plants formed typical root-nodules.

Bacillus tuberigenus (No. 3).—Motile, and 0.3 to 0.6 μ in size. On gelatine, well-defined, yellowish-brown, finely granular colonies develop, the medium being rapidly liquefied. A bright reddish-brown coating forms on potatoes.

Bacillus tuberigenus (No. 5).—Non-motile and more slender than No. 3 (0.25 to 2 μ).

On Gelatine it forms colonies resembling those of the *B. anthracis*, but does not send offshoots into the surrounding media.

On Potatoes it forms yellow prominent drops.

These bacilli, as well as *B. radicola*, penetrate the root-tissue of the Leguminosæ, and form in the cells irregular masses with offshoots and vacuoles, and as such possess the faculty of assimilating the nitrogen of the air.

THE NITRIFYING BACTERIA.

According to Winogradsky, the many species of nitrifying bacteria can be classified into two groups—*Nitroso-bacteria* and *Nitro-bacteria*. The *Nitroso-bacteria* oxidize ammonia to nitrous acid.

During the oxidation processes the bacteria are protected from injury by the presence of bases which take up the acids with which the ammonia was initially combined, and also neutralize the resulting nitrous or nitric acid. Calcium carbonate performs this function excellently in the soil. Free alkali is unsuitable here for the fixation of the acids, because if present in quantity it would be injurious to the bacteria. In artificial cultures Winogradsky replaces the calcium carbonate by magnesium carbonate (Lafar's *Tech. Mycology*, § 204).

Nitrosomonas europæa (Winogradsky).—Found in all samples of European, African, and Japanese earth examined.

Microscopical Appearances.—Short chains of three to four individuals.

Spore Formation not observed.

Motility present, one flagellum.

Biological Characters.—For method of isolating this organism, see p. 71.

On Silicic Acid Media the colonies are at first compact, with a sharp contour, brownish colour, sometimes resembling a spindle with blunt ends. In ten to fourteen days, round, clear, unstained masses, with irregular offshoots, consisting of motile organisms, extend outward from the growth.

In Fluid Cultures, when quiescent, they collect in zoöglææ, forming a sediment, especially around the precipitated carbonate. In seven days or less the fluid becomes cloudy, after which, within twenty-four to forty-eight hours, the germs sink to the bottom of the tube, growth and nitrite formation having ended.

Nitrosomonas javaniensis (Winogradsky). — Found in Java earth, and very similar to the above organism.

Nitrosococcus braziliensis.—Found in the soil of Campinas, Brazil. The *Nitrosococcus* does not form zoöglææ, and possesses no cilia. It attains a diameter of $2\ \mu$. The species grown from Melbourne soil is indistinguishable from that found in Brazil; while that obtained from Quito (Ecuador) is a coccus 1.5 to $1.7\ \mu$ in diameter.

Nitro-bacterium (Winogradsky).—This organism was isolated from Quito earth, and forms nitrates out of nitrites.

Microscopical Appearances.—Very small rods, 0.2 to $0.25\ \mu$ by $0.5\ \mu$.

Motility.—Non-motile.

Biological Characters.—On *Silicic Acid Plate-Cultures* they develop in lenticular or club-shaped colonies.

In Fluid Media they develop in the form of thin films, firmly adherent to the walls and bottoms of the flasks; there is no cloudiness.

Both nitroso- and nitro-bacteria are always present in the soil, the latter immediately oxidizing the nitrous acid generated (from the ammonia salts) by the former. Whether nitrification commences in the dung-heap or in the field is dependent on various circumstances. It takes place whenever a sufficient amount of salts of ammonia has been produced by the fermentation of urea, provided there is a free access of air. Immendorff showed that in the outer layers of manure-heaps (especially horse-dung) nitrous acid is produced in a few days. On account of the formation of easily lixiviable nitrates, which may, moreover, expose the material to wasteful reduction processes, endeavours should be made to minimise the aëration of the manure by battening the heaps well down. It is well known that the soil has no power of fixing nitrates, as according to Dehérain and others a certain portion of the added saltpetre invariably escapes in the drainage water, so that more has to be added to the soil than is recovered in the crop. In manuring with salts of ammonia no such waste occurs, as they are fixed by the soil and protected from wasteful lixiviation, the nitrifying bacteria then oxidizing the ammonia and supplying the plant with nitrites according to its requirements (Lafar's *Tech. Mycology*, §§ 206, 208).

PART IV.

The Hyphomycetes, or Mould Fungi.

ACHORION SCHÖNLEINII.

THIS fungus, the cause of favus in man, horses, cattle, dogs, cats, rabbits, and mice, was discovered by Schönlein in 1839. It is found in the so-called favus crusts. According to Neebe and Unna, there are nine varieties of favus produced by as many different species of Achorion. The disease is most frequently observed on the hairy parts of the head, less often on skin devoid of hair. The fungus causes a loss of hair, as it penetrates the hair and also the hair-sheaths. It also attacks the nails (Onychomycosis), the parasite being located between the cells of the epidermis and the corium. Kaposi has described a case of favus universalis.

Microscopical Appearances.—When a favus crust is rubbed up in 5 per cent. caustic potash and examined microscopically, the mycelium and spores of the fungus are observed lying amidst swollen epithelial cells. The mycelium is more developed, as also broader and more branched than in *Trichophyton tonsurans*.

Biological Characters.—The growth takes place at both room temperature and at 37° C. on all the ordinary nutrient media, though best on agar. In the beginning the growth is white, later it becomes yellow, and fine offshoots penetrate the medium. (See Fig. 115.)

On Gelatine Plates, white, stellate colonies, with thick centres. The deep colonies appear first. The medium acquires a yellow colour, and is slowly liquefied.

In Gelatine Stab-Cultures the appearances are similar, the gelatine being however only softened, not liquefied.

On Agar a puckered whitish coating is rapidly formed, the under surface being yellow, and the edges moss-like. (See Fig. 114.)

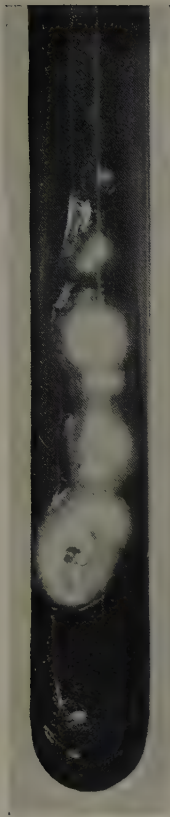


FIG. 114.—*Achorion schönleini*.
Agar culture.



FIG. 116.—*Trichophyton tonsurans*.
Agar culture.

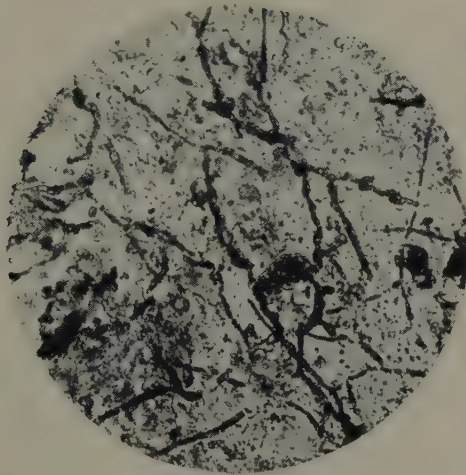


FIG. 115.—*Achorion schönleini*. Section through an agar culture.
Fuchsin. $\times 600$.

It forms oidia after about the fifth day in culture, the threads breaking up into oval bodies, which subsequently separate.

Pathogenesis.—Infection can be produced artificially with material containing spores.

DERMATOMYCES GALLINARUM (SCHÜTZ).

Chicken favus, or *Tinea galli*, is due to a fungus which attacks the comb, wattles, and side of the throat. Round, whitish spots appear, which usually become confluent, spreading to the neck, breast, and body.

Microscopical Appearances.—The fungus consists of a mycelium formed of pointed and often branched threads of variable dimensions, which often have small wart-like pedunculate projections, while other joints are club-shaped and sometimes found free, and here and there fringed with offshoots. In some cases, fine offshoots can be seen on the sides of the mycelium bearing one or two club-shaped grey-coloured bodies.

Biological Characters.—On *Gelatine* a whitish growth develops; the gelatine is liquefied, and acquires a reddish colour.

It also grows on potatoes and bread-paste, the best temperature being about 30° C.

Pathogenesis.—The characteristic symptoms are produced in chickens with pure cultures; white mice, rabbits, and various other experimental animals remain unaffected. According to the conclusions of Constantine and Subrayes, three distinct parasites are the cause of favus in man, the dog, and the fowl. Human favus is nearly related to that of the dog, but distinguished from the latter by its appearance in cultures, its mycelium and colour.

TRICHOPHYTON TONSURANS (GRUBY).

This fungus is the cause of *herpes tonsurans*. It grows in the skin which is covered by hair, and penetrates through the hair, rendering it brittle. In severe cases it may penetrate deeper, and lead to suppuration, and may also affect the nails (*Onychomycosis trichophytina*).

Microscopical Appearances.—The mycelial threads are less broad than those of *A. schönleini*, and do not show much tendency to

branch. Conidia are given off from some of them, as in *Oidium lactis*. (See Fig. 117.)

Biological Characters.—Though it grows at room temperature, the optimum temperature is 30° C.

On Gelatine Plates.—Semi-globular, white, later yellow, liquefying colonies.

In Gelatine Stab-Cultures the growth at first resembles that of anthrax; later a white coating is formed, which floats on the surface when liquefaction occurs.

On Agar it forms white, somewhat puckered, tufts (see Fig. 116); underlying portions are of a yellowish colour.

On Blood-Serum, white tufts; the medium is liquefied.

On Potatoes the growth varies considerably, and is not typical. In fact, the differences noted in the growth on potatoes have led some authors to claim the existence of a number of species. Some authorities, however, claim that the differences are due to variation of a single species.

Pathogenesis.—The cultures retain their vitality for a long time. Herpes tonsurans has been reproduced experimentally with material containing gonidia.

THRUSH.

This disease is caused by a fungus, the *Oidium albicans*, and occurs on all mucous membranes with squamous epithelium, especially in the mouths of enfeebled infants. The disease begins in the tongue, slightly raised, pearly-white spots appearing, which spread and coalesce. The disease then spreads to cheeks, lips, etc.; in bad cases the whole buccal and œsophageal mucous membrane is affected. The membrane which is formed may be easily scraped off.

Microscopical Appearances.—Sometimes mycelial threads are present; at other times round or oval conidia, resembling yeast cells.

Biological Characters.—Aërobic; optimum temperature, 37° C.

On Gelatine Plates, white, concentric, non-liquefying colonies, with raised margins.

In Gelatine Stab-Cultures, yellowish-white growth, with processes extending into the medium.

On Agar, a yellowish-white growth.



FIG. 117.--*Trichophyton tonsurans*, from agar culture. Unstained. $\times 350$.



FIG. 118.—*Oidium lactis*, from gelatine culture. $\times 1000$.

On Potatoes, a thick, white coating, which subsequently turns yellow, the surface being grooved and the edges irregular.

On Bread-Paste, a thin, white coating.

Pathogenesis.—Pathogenic for rabbits when inoculated intravenously, the fungus becoming localised in the internal organs. Roger (1896) produced immunity to the infection in rabbits by inoculating them with increasing doses of culture.

OIDIUM LACTIS.

Found in sour milk, on bread, and decayed fruit. On cream the colonies can be recognised by transmitted light as faintly yellow round spots. Formerly confused with *O. albicans*.

Microscopical Appearances.—The branched mycelial threads are segmented and give off colourless hyphæ; undergo transverse segmentation, giving rise to terminal oidia. (See Fig. 118.)

Staining Reactions.—Stains easily with the ordinary anilin stains; but, owing to the heating in the preparation of ordinary dried specimens, the organisms appear shrivelled.

Biological Characters.—It grows well on all the ordinary nutrient media, especially when the reaction is slightly acid. The optimum temperature lies between 15° to 20° C.

On Gelatine Plates, a white, long, hairy, mycelial growth develops, which covers the plate but does not liquefy the medium.

On Agar the growth at first is delicate, but later a viscous, yellowish-white, folded coating forms.

On Potatoes, a whitish, velvety growth.

On Milk a pellicle is formed, the medium not being visibly affected.

Non-pathogenic.

PENICILLIUM GLAUCUM.

This fungus is of universal occurrence. It is found on bread, jam, cheese, decaying fruit, barley, and on the walls and ceilings of rooms. At first whitish tufts appear, which later acquire a green colour, due to the formation of myriads of spores.

Microscopical Appearances.—The mycelium gives rise to fruit-hyphæ, which branch dichotomously and form terminal chains of spores. (See Fig. 119.)

Biological Characters.—It grows best at room temperature on any

of the ordinary media. It is also able to propagate itself sexually when placed under certain conditions, especially in the absence of oxygen.

The spores will germinate at any temperature between 2° and 43° C.; 22° to 26° being most favourable. According to Pasteur, dry spores withstand a temperature of 108° C., but are soon killed when immersed in boiling water.

It is non-pathogenic.

THE ASPERGILLI.

The *Aspergilli* produce conidia from so-called sterigmæ, arising close to one another upon the swollen extremity of the fruit-hyphæ. The conidia are easily detached, and are capable of giving rise to a fresh mycelium if they fall upon a suitable medium. This is the usual mode of reproduction. Under certain circumstances the *Aspergilli* may also undergo sexual reproduction.

Asp. nidulans.—Found on bread, on which it forms light-green tufts. Optimum temperature, 40° C. On potatoes and bread it forms a reddish-brown pigment, which penetrates the medium. *Pathogenic* properties similar to those of *A. fumigatus*.

Asp. niger.—Blackish-brown mycelium and conidia; optimum temperature, 34° C. *Pathogenic.* (See Fig. 120.)

Asp. ochraceus.—Yellowish-red to dark-yellow tufts.

Asp. oryzae.—Found on rice; at first flesh-coloured, later of an ochre-yellow colour; changes starch and dextrin into sugar. Used in the preparation of the Japanese rice-spirit.

Asp. repens.—Found on fruits preserved with sugar. Appears at first white, later as greenish tufts, with smooth, colourless or greenish spores.

Asp. subfuscus.—Found on bread. Appears as tufts, which are yellowish-green to olive-black in colour. Optimum temperature, 37° C. *Pathogenic.*

Asp. albus.—Whitish tufts.

Asp. clavatus.—Greenish tufts; club-shaped fruit-bearers on long hyphæ; small conidia.

Asp. flavus.—Found in bread; greenish-brown tufts, and yellowish-

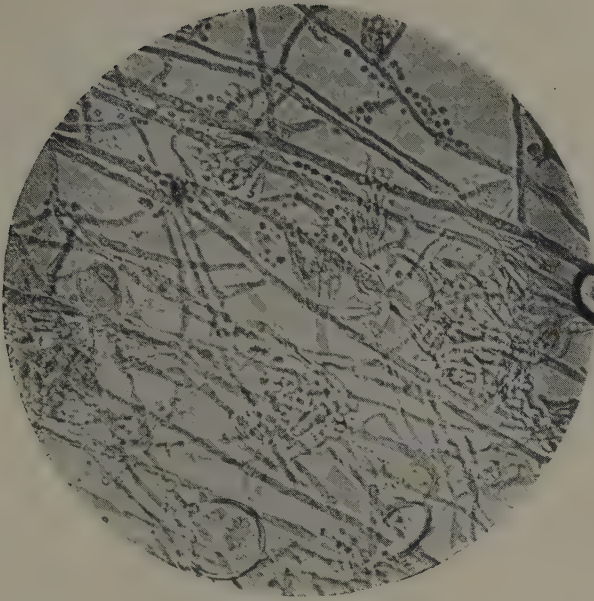


FIG. 119.—*Penicillium glaucum*, from gelatine culture. Unstained. $\times 300$.

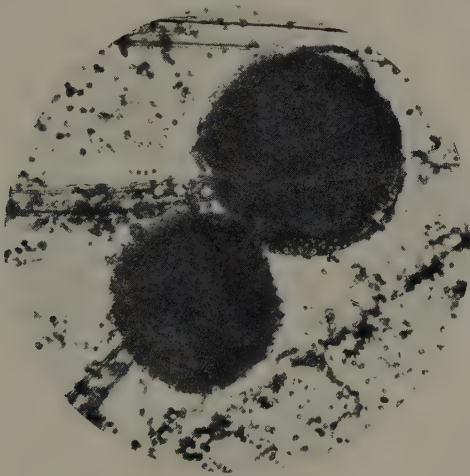


FIG. 120.—*Aspergillus niger*, from agar culture. Unstained. $\times 350$.

brown spores. Optimum temperature, 28° C. The *pathogenic* properties are the same as those of *Asp. fumigatus*.

Asp. fumigatus.—Found in the trachea and bronchi of birds, and also on bread; bluish-green tufts, which later acquire a bluer or ashy colour; very small smooth spores. Optimum temperature, 37° to 40° C. (See Fig. 121.)

Pathogenesis.—When rabbits and dogs are injected intravenously with the spores, death occurs in about twenty-four hours. The mycelium is found in all the organs, especially in the substance of the heart and kidneys. The lungs (*Pneumomycosis aspergillina*), auditory canal, and cornea may be affected in man.

Asp. glaucus.—Found in fruit, cabbage, and on damp wooden walls. Greenish-coloured tufts and round spores with rugged surfaces. Optimum temperature, 10° to 15° C. *Non-pathogenic*.

THE MUCORS.

In the mucors the spores are formed within a spherical sporangium situated upon the extremity of the hypha. When the sporangium has attained a certain size its protoplasm divides into a number of spores, and when these have ripened they are liberated through rupture of the sporangial membrane. Falling upon a suitable medium the spores give rise to a new mycelium.

Mucor corymbifer.—Occurs as greyish tufts. The hyphæ are branched and the sporangia colourless. The spores measure 3μ to 2μ . Optimum temperature, 37° C.

Pathogenesis.—When injected into the veins of rabbits, death follows in from two to three days. The fungus is mostly found in the kidneys and lymph-follicles of the intestinal mucosa, where it forms a mycelium. Dogs are immune. (See Fig. 122.)

Mucor mucedo.—Found on horse-manure as a whitish growth, like thistledown. The hyphæ are 1 to 13 cms. in length, and may be single or branched. Crystals of oxalate of lime occur on the outside of the sporangia, which when mature are coloured brown or black. Optimum temperature, 37° C. *Non-pathogenic*. (See Fig. 123.)

Mucor pusillus.—Found on moist bread; occurring in tufts, which are at first white, later grey. The mycelium is unusually fine. The

sporangia are covered with a spinous membrane. Optimum temperature, 45° C.

Pathogenesis.—Same as *Mucor corymbifer*.

Mucor ramosus.—Occurs on moist bread as tufts, at first white, and later brownish. The mycelium is very much branched. The hyphæ are long and branched; large spores. Optimum temperature, 40° C.

Pathogenesis.—Same as *Mucor corymbifer*.

Mucor racemosus.—Found on sugar and starchy substances. Fruit-hyphæ not exceeding 1 to 5 cms. in length; numerous and delicate. Sporangia are yellow or yellowish-brown; round spores.

Mucor rhizopodiformis.—The mycelium is at first quite white, and later turns greyish. The hyphæ are twisted; at first ascend, and then grow down again into the medium, into which they send down root hairs. Spores colourless; diameter, 5 to 6 μ .

Mucor stolonifer.—The mycelium has branches which at first ascend and then descend, and are covered with fine root hairs. The sporangia are deep black and warty; brown spherical spores, 10 to 20 μ in diameter.

Pathogenesis.—Same as *Mucor corymbifer*, from which it is distinguished by the pleasant, fruity smell of the cultures.

FUSISPORIUM MOSCHATUM (KITASATO).

This fungus was found as an accidental growth in a vegetable infusion. The principal characteristics are the formation of sickle-shaped spores (see Fig. 124), and an odour of musk given off by the cultures.

Biological Characters.—It grows on all the ordinary media, on rice paste, and infusion of peas, but only at room temperature.

Gelatine is liquefied slowly; the cultures at first are greyish-white, eventually becoming rose or brick-red coloured.

In preparing and mounting microscopic specimens of the various fungi figured in this work, the author adopted the following method:—

1. Hammer out a piece of platinum wire quite flat at the point, in the shape of a shovel.

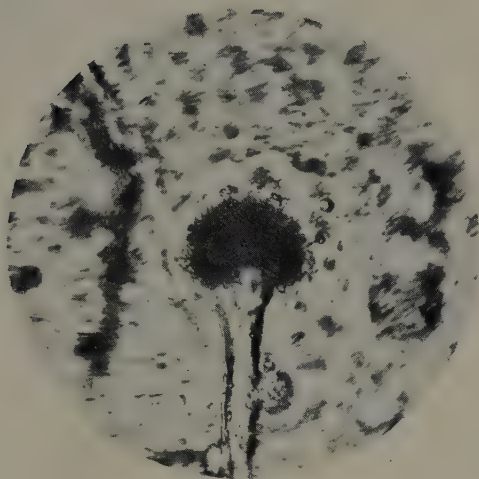


FIG. 121.—*Aspergillus fumigatus*, from agar culture.
Unstained. $\times 350$.

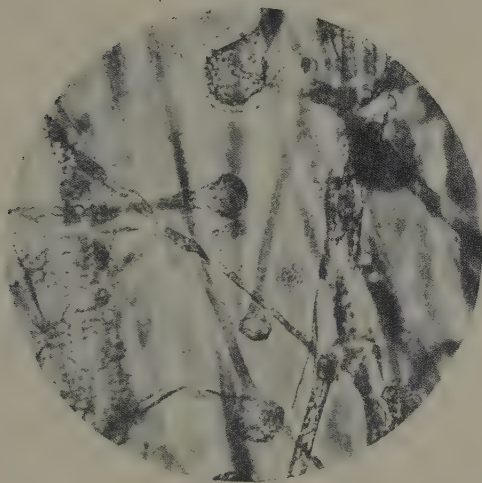


FIG. 122.—*Mucor corymbifer*, from potato culture.
Unstained. $\times 350$.

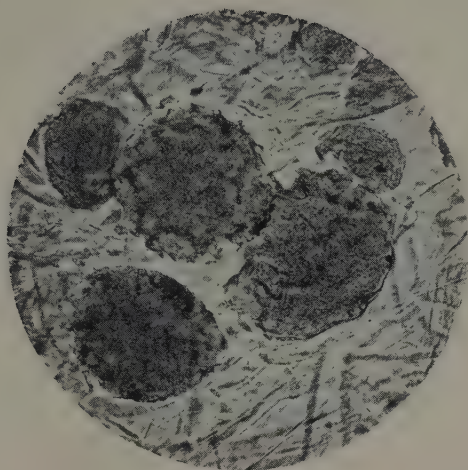


FIG. 123.—*Mucor mucedo*, from gelatine culture, showing Zygotes
Unstained. $\times 350$.

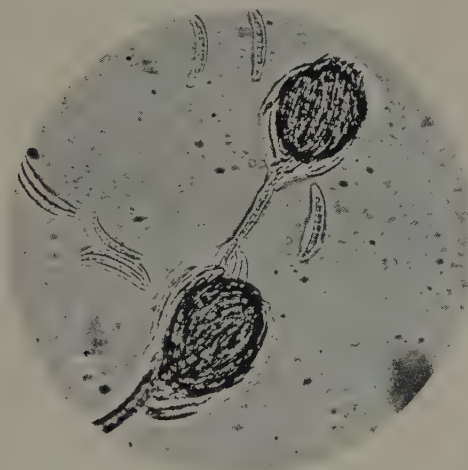


FIG. 124.—*Fusisporium moschatum* from potato culture,
showing sickle-shaped spores. Unstained. $\times 350$.

2. Select an isolated growth, heat the shovel, and cut through the medium a short distance from the growth.
3. Push the platinum spade under the growth, and transfer the mass *en bloc* to a clean cover-glass.
4. Place the cover-glass on a clean slide, heat gently over the flame (just enough to melt the medium), and press the cover-glass gently to remove superfluous medium.

By this process the fungus is mounted in the medium in which it is growing. In making permanent mounts by this method, a drop of formalin solution is added before the slide is heated.

PART V.

The Blastomycetes, or Yeasts.

THE yeasts are divided into two groups :—

(a) *Saccharomycetes*, or true yeasts, in which true *spore-formation* occurs.

(b) *Torulæ*, in which *no spore formation* has been observed.

The Blastomycetes reproduce themselves by gemmation or budding, which distinguishes them from the Schizomycetes or Bacteria, which reproduce themselves by fission. The Blastomycetes are distinguished from the Hyphomycetes by being unicellular and by their asexual reproduction. The yeasts employed for commercial purposes consist of a mixture of different species. We are indebted to the researches and experiments of Hansen for our present knowledge of the various changes produced by the different species during fermentation, as well as for the method of isolating them by means of plate-cultures. The various species may be distinguished by :—

1. The temperature at which ascospores develop.
2. The character of the film forming on the surface of the fluid during fermentation.
3. The sugars which are fermented.
4. Whether the variety under observation causes a top or bottom fermentation.

METHOD OF OBTAINING PURE CULTURES.

1. A Pasteur flask containing the wort to be experimented with is placed in the thermostat.
2. A given quantity of sterilized water is added to the developed culture, and the yeast cells in a drop counted under the microscope.
3. Supposing ten cells to be present, a similar-sized drop is now

transferred to a flask containing 20 c.c. of water, which is equivalent to one yeast cell for each 2 c.c. of water.

4. The flask containing the 20 c.c. of water with the ten yeast cells is thoroughly shaken, and this liquid divided equally, 1 c.c. being placed in each of twenty flasks containing sterilized wort.

5. If the separation has been complete, ten out of twenty flasks should contain one organism each, but this of course cannot be absolutely depended on.

6. At this stage Hansen shakes the flasks very vigorously to separate the cells as much as possible, and places the flask in the incubator, allowing them to remain perfectly still, in order that the cells may sink to the bottom or become attached to the walls of the flasks.

7. At the end of several days the flask is carefully lifted and examined, and it is noted whether one or more white specks have been formed on the walls of the glass; if only one such speck is found, *it is a pure culture* (a single colony).

This method is especially useful when the yeast plants are weakly. Vigorous species can be isolated from mixtures by means of wort-gelatine plate-cultures. (For the methods of obtaining pure cultures on a large scale, see the works of Hansen and Jörgensen.)

SACCHAROMYCES CEREVISIÆ I.

This is known as the Old English top-fermentation yeast, and is used by brewers and bakers.

Microscopical Appearances.—Large round or oval cells, most frequently 6 to 8 μ in diameter. In the earlier stages of film-formation delicate mycelial-like threads are formed, which, as the film becomes older, grow longer and more regular. Nuclei can be demonstrated in the cells, especially in old cultures, when stained with hæmatoxylin or osmic acid. The cells are sometimes very granular.

Spore-Formation.—Ascospores develop after twenty-four hours at 10° to 37° C., but most rapidly at 30° C. For the development of the ascospores, Hansen employs plaster-of-Paris blocks, which are first sterilized by heat. A particle of yeast is placed upon the upper surface of the block, the lower surface resting in a small vessel containing water, within a sterile air-chamber, the apparatus being placed in an incubator, or left at room temperature. Ascospores can also be grown on potatoes prepared according to Globig's method. (See p. 61.)

The author has found the following simpler arrangement to yield most satisfactory results. An oblique plaster-of-Paris block is prepared, of a shape similar to the sliced potatoes used in ordinary potato-cultures in tubes. The plaster is cast in a wooden mould of suitable shape, the inside of the mould having been rendered non-adhesive by the application of melted paraffin. The plaster block rests on the bottom of an ordinary test-tube, about 1 in. in diameter, containing a little water in the bottom. The tube is then plugged and sterilized. Some yeast is placed on the upper portion of the oblique surface of the plaster block, after which the tube can be placed in the incubator or left at room temperature.

Staining Reactions.—Dried specimens can be stained with fuchsin and methylene-blue. This stain is also used to differentiate living and dead cells in hanging-drop cultures, the latter alone staining. The author has found that ascospores can be beautifully demonstrated by the Claudius method of staining (p. 21), the cells being stained blue, and the spores and background remaining yellow (see Fig. 126). Ascospores can also be stained by the ordinary method for staining spores (see pp. 33 and 34).

Film-Formation.—This takes place most rapidly (seven to ten days) at a temperature of from 20° to 22° C., most slowly (two to three months), at 6° to 7° C., and ceases altogether at temperatures above 38° C. and below 5° C.

Biological Characters.—On *Gelatine Plates* it forms small white colonies. The individual yeast cells which form the surface colonies can be distinguished by means of a low power.

This yeast converts saccharose (cane-sugar) into invert sugar, which is fermented, as are also dextrose and maltose, alcohol and carbonic acid gas being formed. Fermentation is accompanied by evolution of heat and great multiplication of the yeast cells. Lactose (milk-sugar) does not seem to be altered.

SACCHAROMYCES ELLIPSOIDEUS I. (HANSEN).

This is a "wild" species of wine ferment. It is found on the surface of fruit, chiefly on grapes.

Microscopical Appearances.—Round or oval cells, which sometimes assume a sausage form.

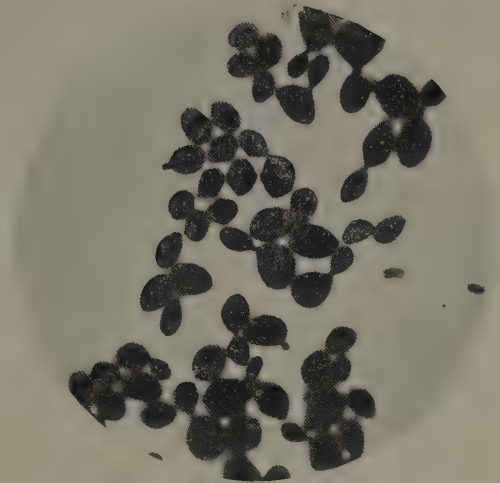


FIG. 125.—*Saccharomyces cerevisiae*, showing budding cells. Potato culture. $\times 1000$.

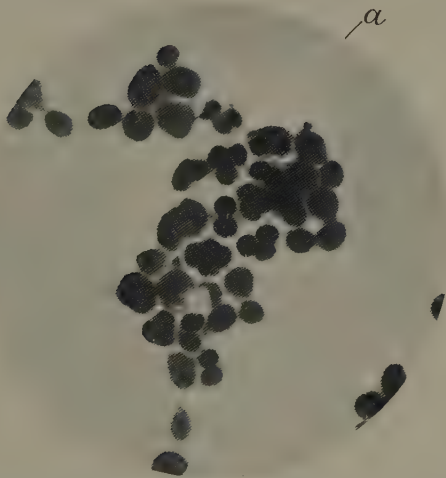


FIG. 126.—*Saccharomyces cerevisiae*. Culture on plaster surface. Stained with carbol-fuchsin and methylene-blue. (α) Ascospores. $\times 1000$.

Spore-Formation.—The spores are from 2 to 4 μ in diameter, two to four being found in a single ascus. They are developed at temperatures between 7.5° and 31.5° C., most rapidly (in twenty-four hours) at 25° C.; above 32.5° C. and under 4° C. the development ceases.

Film - Formation.—The surface membrane, always delicate, is formed most rapidly (eight to twelve days) at 33° to 34° C.; most slowly (sixty to ninety days) at 6° to 7° C. Development ceases under 5° C. and above 38° C. The growth is most characteristic between 13° to 15° C., when it consists of a complicated branching mass of elongated cells or threads, arranged in rows with lateral processes coming off at the point of junction. Secondary branches are formed at the constrictions of the primary branches.

On Wort-Gelatine the colonies present a net-like appearance. It causes as powerful and rapid fermentation of the various carbohydrates as *S. cerevisiae*.

SACCHAROMYCES ELLIPSOIDEUS II. (HANSEN).

A "wild" or wine-fermentation yeast, which gives rise to the muddiness of beer.

Microscopical Appearances.—In young cultures at 15° C. the cells are usually somewhat rounded or egg-shaped; older cultures exhibit longer mycelial rods with forked transverse shoots given off at the joints.

Spore-Formation.—The spores are from 2 to 5 μ in diameter, two to four being found in a single ascus, and may be egg-shaped, slightly irregular, or elongated. They are developed most rapidly at 29° C., most slowly at 8° C.; above 34° C. and below 4° C. the development ceases.

Film-Formation.—It is essentially a low yeast, and the film that forms is very delicate. At 33° to 34° C. it appears in three to four days, but not for five to six months at 3° to 5° C. At 2° and at 40° C. no film is developed.

SACCHAROMYCES PASTORIANUS I. (HANSEN).

A "wild" yeast, the spores frequently occurring in the atmosphere of breweries. It gives an unpleasant bitter taste and a bad smell to beer.

Microscopical Appearances.—It occurs as elongated ellipsoidal or pear-shaped cells, from which small apical or lateral branches are sometimes given off.

Spore-Formation.—The asci are usually elongated or rounded, and may contain two or up to eight or even more spores, which vary in size from 1.5 up to 5 μ . They are developed most rapidly (seven to ten days) at 27.5° C., most slowly (fourteen days) at 3° to 4° C. The development ceases at .5° C. and at 31° C.

Film-Formation.—The films, which are usually very delicate, are developed most readily (seven to ten days) at from 26° to 28° C., most slowly (five to six months) at from 3° to 5° C. ; development ceases at 34° and 2°. Mycelial-like threads develop freely in the film at from 3° to 15° C., and most irregular forms appear. In the older films numerous irregular club, skittle-shaped, and other forms occur. In the younger films the cells are usually smaller and the irregular forms less frequent.

SACCHAROMYCES PASTORIANUS II. (HANSEN).

This was also isolated from the air of a brewery. It is a feeble top-fermentation yeast when growing in beer-wort. It gives rise to neither cloudiness nor to any unpleasant bitter taste.

Microscopical Appearances.—The sedimentary cells are mostly elongated, but may be slightly rounded, varying considerably in size. The cells found in the film are rounded, egg-shaped, or somewhat elongated.

Spore-Formation.—The asci are usually elongated, the spores occurring in multiples of two, and measuring from 2 to 5 μ in diameter. They are developed most rapidly (twenty-seven hours) at 23° C., most slowly (seventeen days) at 3° to 4° C. ; formation ceasing at 29° C. and at .5° C. This yeast secretes an invertase, and causes fermentation of all the carbohydrates that are fermented by the other yeasts of this group. In old cultures of the films the cells are small, thread-like, and very irregular in shape.

SACCHAROMYCES PASTORIANUS III. (HANSEN).

According to Hansen this yeast is one of the causes of turbidity in beer.

Microscopical Appearances.—The cells are very similar to those of the sedimentary yeast, but at a temperature of from 15° down to 3° C.

elongated mycelial-like threads develop, which in old cultures become still more characteristic. In *S. pastorianus* I. the mycelial threads are most characteristic at 13° to 15° C., while at 15° to 3° C. the cells in *S. pastorianus* II. are oval and rounded.

Spore-Formation.—Similar to that of *S. pastorianus* II. Spores form most rapidly (twenty-eight hours) at 25° C., most slowly (nine days) at 8.5° C., and sporulation ceases at 29° C. and at 4° C.

Film-Formation.—This appears in the form of small flakes; most rapidly (seven to ten days) at 26° to 28° C., most slowly (five to six months) at 3° to 5° C., and ceases altogether at 34° and 2° C. The elongated or sausage-shaped cells predominate, but large and small round and ovoid cells are also found in the sediment and in the films at from 20° to 28° C.

Biological Characters.—In cultures grown on yeast-water gelatin, at the end of sixteen days the colonies exhibit peculiarly fringed edges. Grown in wort, this yeast gives rise to a top-fermentation, causing considerable turbidity, and producing alcohol and carbonic acid gas.

"SACCHAROMYCES" APICULATUS.

Found in fermented wine and spontaneously-fermented beer, and in hot seasons on sweet, succulent fruits—cherries, plums, grapes, etc. In winter it is found in the soil beneath the trees that bear these fruits.

Microscopical Appearances.—In fresh fluid cultures the cells and buds are lemon-shaped, in older cultures they are oval.

Spore-Formation.—Not observed, hence it should be classified under *Torula*.

Biological Characters.—When dried in a thin layer it is killed, which accounts for it not developing on unripe fruit. It is a bottom-fermentation yeast, producing but little alcohol. It does not invert cane-sugar, but acts on dextrose in yeast-water, the fermentation being incomplete.

SACCHAROMYCES ANOMALUS.

Found in brewery yeast. Small oval cells. Spores occur in the form of hemispheres with projecting rims (shaped like a hat). The optimum temperature for their development is 25° C.

SACCHAROMYCES MARXIANUS.

First found in wine.

Microscopical Appearances.—Small ellipsoidal, ovoid, or occasionally sausage-shaped cells, often arranged in colonies.

Spores are not freely developed. They are more frequent on solid media, and are usually oval or kidney-shaped.

Film Formation takes place very slowly, the film consisting of oval and short sausage-shaped cells.

Biological Characters.—In beer-wort it is not very active. It does not ferment maltose, but acts strongly on saccharose, which it inverts, and then ferments with great rapidity. It also acts upon dextrose.

SACCHAROMYCES MEMBRANÆFACIENS.

Forms a bright-yellow tough scum on beer-wort, being composed of long and sausage-like cells, occurring either singly or closely packed together. Spores are formed rapidly. Nutrient gelatine is liquefied. It does not cause any fermentation of ordinary carbohydrates, and does not invert cane-sugar.

SACCHAROMYCES EXIGUUS.

Found in German yeast by Hansen. It forms no mycelial threads on beer-wort or on solid media. It forms spores, but sparsely. The *film* is very delicate, and consists of short rod-shaped or ovoid cells. It causes the same changes in sugars as the *S. marxianus*.

SACCHAROMYCES ACIDI LACTICI.

Cells ellipsoidal. It forms white, glistening colonies on gelatin plates and slanted agar; a brownish coating on potato. Milk is coagulated, an acid being formed. In solutions of milk-sugar it forms alcohol.

MYCODERMA CEREVISIÆ ET VINI.

A number of species are included under the above name, some of them causing slight alcoholic fermentation.

"Copenhagen" forms variously-shaped cells of a more transparent and less refractive appearance to those of true *Saccharomycetes*.

Hansen describes a form of *M. cerevisiæ* occurring in long cells, which do not transmit light so strongly as the *Saccharomycetes*.

Biological Characters.—On wort-gelatine, small, dim, light-grey colonies, which either spread over the surface or cause a shell-shaped cavity in the medium.

On Wort or Beer it forms a greyish-white thickly-matted film. Develops at 2° to 15° C.

Actions.—It does not cause alcoholic fermentation, nor does it invert cane-sugar.

THE TORULÆ.

The *Torulæ* are widely distributed yeast-like organisms, of a spherical or elongated form. As shown by Hansen, they are distinguished from the genus *Saccharomyces* by their inability to form endogenous spores. They multiply by budding, and in some cases by mycelium formation. The presence of a mycelium in certain forms suggest that they may be derived from the higher fungi.

Though some *Torulæ* produce alcoholic fermentation, most of them exert but a feeble fermentative action or no fermentation. Outside of numerous forms described by Hansen and others as occurring in breweries (and some of these injure the product), *Torulæ* which produce pink, white, or black colonies, etc., are not infrequently isolated from the air.

Pathogenic Blastomycetes.

“SACCHAROMYCES HOMINIS.”

Found in an infectious disease, which began with a subperiosteal inflammation of the tibia, terminating in chronic pyæmia.

Microscopical Appearances.—Round or oval double-contoured cells possessing capsules.

Biological Characters.—*On Gelatine Plates*, prominent, round, non-liquefying colonies.

On Agar, a white coating.

On Potatoes, a greyish-brown coating.

On Blood-Serum, a drop-like growth.

Bouillon is clouded, a film growing on the surface.

In Grape-Sugar Bouillon fermentation takes place, alcohol and CO₂ being formed.

Pathogenesis.—In rabbits a local abscess forms. Mice die from septicæmia.

SACCHAROMYCES LITOGENES.

Found in the lymph-glands of an ox affected with carcinoma of the liver. Large and small round cells with a membrane. The growth in the various media is similar to that of *S. neoformans*, except on *potatoes*, on which a dark-brown coating develops.

Pathogenesis.—In guinea-pigs a tumour forms at the point of inoculation and nodules in the organs. The yeast cells in the centre of the nodules very frequently degenerate.

SACCHAROMYCES NEOFORMANS.

Found in the juice of fruits. Round or elliptical double-contoured cells, containing refractive granules.

On Gelatine Plates, round, cup-shaped, non-liquefying colonies are formed. *In Gelatine Stab-Cultures* a granular growth occurs along the track of the needle.

On Agar a dry film develops. *On Potatoes*, a white elevated growth. *Milk* is not coagulated.

In Sugar-Bouillon a sediment forms, and often a film is developed.

Pathogenesis.—In guinea-pigs a tumour forms at the point of inoculation, and nodules develop in the internal organs.

SACCHAROMYCES SUBCUTANEUS TUMEFACIENS.

Found in a myxomatous tumour of the upper part of the thigh.

Oval or round cells, frequently possessing large transparent capsules. *In Gelatine Stab-Cultures* the development occurs in small colonies; it causes no liquefaction of the medium. *On Agar* there is a thick creamy growth.

On Potatoes an extensive white coating forms, which later becomes brown. *On Acid Beer-wort Agar*, a brown coating. *In Alkaline Bouillon*, a slight sediment. *In Beer-wort*, a thick sediment without film-formation.

It slightly ferments saccharose.

Pathogenesis.—White mice and rats are susceptible, extensive local tumour being produced.

"BLASTOMYCES DERMATITIDIS."

Isolated in pure culture by Gilchrist and Stokes (1898) from a man suffering from a disease similar to lupus. Cultivation succeeded on all media ; best on potato, on which it occasionally exhibited a mycelial growth and conidia. It did not ferment sugar. The germ was proved to be pathogenic by inoculations on horses, sheep, dogs, and guinea-pigs.

PART VI.

The Protozoa.

AMŒBA COLI.

FOUND in the human intestines and stools in cases of dysentery, and abscess of the liver associated with dysentery.

Microscopical Appearances.—Ovoid, elongated, or irregular amœboid organisms, measuring usually 25 to 35 μ . When at rest, the Amœbæ frequently assume a spherical or ovoid form. When viewed on a warm stage, they may be observed to move about, sending out blunt, hyaline pseudopodia. The endoplasm is granular, the ectoplasm hyaline. Vacuoles are frequently present in the endoplasm, as well as foreign bodies, red blood-corpuscles, and bacteria. The large nucleus (6 to 8 μ), with a nucleolus, is rendered plainly visible in stained specimens, or by the addition of acetic acid. The Amœbæ have been observed to multiply by division. They die within twenty-four hours in the dejections.

All attempts at cultivation have up to the present failed. The *Amœba coli* found in the normal intestinal contents cannot be distinguished morphologically from that found in dysentery; but the latter, when introduced into the rectum of cats, causes an ulcerative hæmorrhagic inflammation of the large intestines (Kartulis). The Amœbæ of dysentery are genuine parasites, which penetrate deep into the submucous, sometimes as far as the serous, coating of the intestines (Councilman and Lafleur).

Method of Examining Stools.—The stools should be examined in a fresh state. It is best to collect them in a vessel previously warmed to body-heat. A drop of dejection is best examined directly under the microscope, unstained, using Nuttall's microscope-thermostat (see p. 16), or a warm stage. In sections, satisfactory prepara-

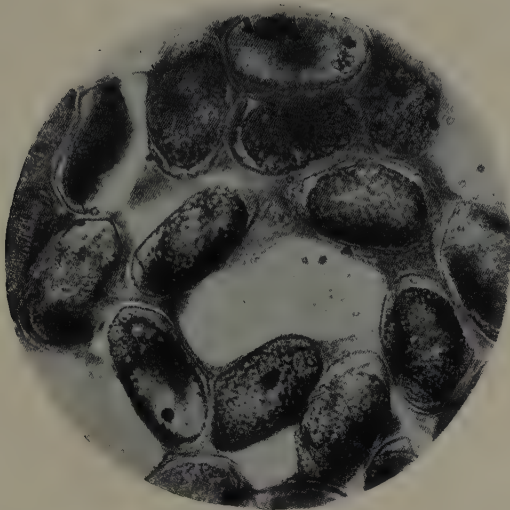


FIG. 127. *Coccidium oviforme*. Cover-glass specimen from liver of rabbit. Stained by Ehrlich's method. $\times 750$.

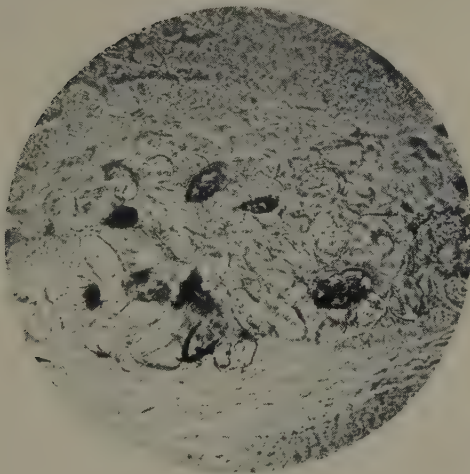


FIG. 128.—Section of a rabbit's liver, showing *Coccidia*. Unstained. $\times 300$.

tions may be obtained by staining with safranin, hæmatoxylin, or eosin.

BALANTIDIUM (PARAMÆCIUM) COLI.

Found in the intestines of man.

Microscopical Appearances.—Elliptical infusoria, 60 to 70 μ long, their whole surface being covered with cilia. The mouth is funnel-shaped, the nucleus bean-shaped, and they possess two contractile vacuoles. They multiply by dividing into four and by conjugation, also sometimes becoming encysted. They possibly exert a pathogenic action, having been found in man, in large quantities, in cases of catarrh and ulceration of the intestine. A similar organism is found in normal swine.

COCCIDIA.

The *Coccidia* are unicellular, usually intracellular, animal parasites belonging to the class *Sporozoa*.

The best known *Coccidia* are those found in the rabbit and the fowl. According to Rieck, there are two species—a liver and a bowel species; the former causing peculiar white or abscess-like cavities in the liver (due to local dilatation of the bile-ducts), the other inhabiting the intestines, and causing acute and fatal inflammation. M'Fadyean records an outbreak of intestinal coccidiosis amongst pheasants, as also an outbreak amongst lambs. Zschokke, Hess, and Guillebeau describe a form of dysentery amongst cattle in Switzerland, produced by *Coccidia*, and known as "red dysentery," or "dysenteria hæmorrhagica coccidiosa." It has also been produced experimentally in cattle, through infection with sporulating *Coccidia*.

COCCIDIUM OVIFORME.

This parasite is found in the rabbit's liver and intestine, and may produce a fatal disease (Psorospermiasis), which occasionally occurs as an epizootic. It enters the gall-ducts by way of the ductus choledochus, penetrates into the epithelial cells, and, increasing in size, becomes encysted. The parasite is surrounded by a delicate external membrane and a shining double-contoured inner membrane. The permanent cysts have granular contents and a nucleus. The development does not proceed further in the rabbit's liver, but outside the animal's body. In contact with moisture, the plasma of the cell divides

into four oval mother spores, each of which again divides into two sickle-formed daughter spores. When these sickle-shaped spores gain entrance to an animal's stomach, the membrane of the mother spores is dissolved by the gastric juice, the free spores enter the intestines, and ultimately reach the gall-ducts. All the parasites in an affected liver do not complete the above cycle of development, but sporulate during their growth in the epithelial cells, exhibiting four to fifty sickle-shaped nucleated bodies, which, becoming disseminated, cause general disease of the liver. White particles, consisting of masses of *Coccidia*, are sometimes found floating in the bile. According to Rivolta, the parasite sometimes develops in the epithelium of the gall-bladder.

The parasite stains well with hæmatoxylin and eosin in sections and cover-glass specimens. The author obtained some specimens from a Jack-rabbit's liver in California in 1895. (See Figs. 127 and 128.)

According to Leuckart, the intestinal species is known as *Coccidium perforans*; but that it is a distinct species is not exactly proven, as both forms frequently coexist in the same host, the difference probably arising from the different position of the parasites in the affected animals.

Coccidia have been observed in the liver, kidneys, and pleural exudate, in a few cases in man.

KLOSSIA SOROR (A. SCHNEIDER).

This parasite is frequently found in the kidneys of various species of land and water snails.

Microscopical Appearances.—Cysts containing a large number of mother spores, which divide into four to six sickle-shaped daughter spores. The latter measure 1 to 7 μ , and for a short time exhibit a serpentine movement. The daughter spores often multiply in the epithelial cells of the kidney, causing hypertrophy. They have also been observed in the urine. (See Fig. 129.)

PYROSOMA BIGEMINUM.

This parasite is the cause of a bovine disease, known in the United States as "Southern or Texas Cattle Fever," in Jamaica as "Ixodic Anæmia," in Australia as "Tick or Cattle Fever." The disease has also been observed in Java, South America, Africa, Roumania, and

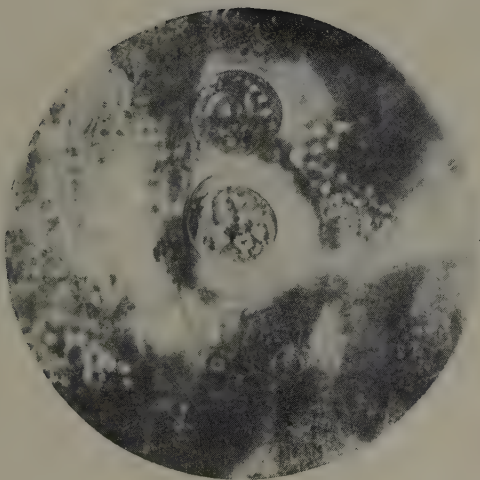


FIG. 129.—*Klossia*. Section of liver of snail (*Aquolimax*).
× 1000.

Italy. The "*Pyrosoma bigeminum*" occurs in the red blood-corpuscles of the affected animals. The affection is commonly known in the United States as "Texas or Southern Fever," from the fact that it is transmitted to northern cattle by animals driven northward from the Southern States during the summer months. A quarantine line, 4000 miles in length, separates the affected from the unaffected districts in the United States, the line extending in 1892 from the Atlantic Coast in the east to the Pacific Coast in the west.

Symptoms.—The incubation period lasts about fifty days. The onset of the disease is indicated by febrile symptoms lasting four to eight days, the temperature rising to 102° to 108° F., the animals becoming sluggish in their movements, and showing a tendency to lie down. There may or may not be diarrhœa (at times hæmorrhagic) and hæmaturia, though the presence of the latter may constitute the first symptom. Pulse and respiration are rapid; there is trembling of the muscles of the neck and posterior parts. The animals walk about with a feeble and tottering gait; the secretions may be suppressed. The animals finally lie down and die. A few may recover, but relapses are frequent, and the disease may last for months. The fatality is greatest in summer, least in autumn. In an outbreak in California, studied by the author in 1888, one rancher lost 700 animals out of a herd of 1200 cattle.

Morphology of the Parasite.—When the blood of an affected animal is examined in the fresh state at ordinary temperature, small bodies are seen inside the red blood-corpuscles. These may be pale rounded masses with amœboid movement and distinct contour, or pear or spindle-shaped bodies, distinctly outlined, with a granular body or vacuole at the thick end. There may be two of the pear-shaped bodies with narrow ends opposed in a single corpuscle. Very rarely, three or four ovoid forms occur in the same corpuscle (see Fig. 130). The bodies measure from 0.5 to $2\ \mu$ in diameter, and also differ from malarial parasites in being unpigmented. When motile, the parasites may be observed to be active for as long as an hour under the microscope. In the circulating blood the infected cells rarely exceed 1 to 2 per cent. In some cases, in late stages of the disease, the parasite may be found free. If an animal dies or is killed in the acute febrile stage, a great number of infected corpuscles are found in the capillaries of the peripheral circulation. The parasites are most plentiful

in the vessels of the kidney, next in the liver, spleen, and heart substance.

Fixed cover-glass specimens are stained with alkaline methylene-blue for one-half to two minutes, washed with water, and placed in a 1 per cent. solution of acetic acid for a few seconds, then washed and examined in water, or dried and mounted in xylol-balsam. The organism can also be stained with a weak solution of gentian-violet. Sections of organs can be stained with fuchsin.

Mode of Infection.—According to Smith and Kilborne, the disease is naturally transmitted by a blood-sucking tick (*Ixodes* or *Boophilus* *bovis*) which lives on the skin of the cattle, and serves as a host of the *Pyrosoma*.

The mature female cattle-tick, when gorged with blood, may be said to resemble a castor-oil bean. It is of a dull lead colour, is rarely more than $\frac{1}{2}$ an inch long and $\frac{5}{16}$ of an inch in breadth, and possesses four pairs of legs. It attaches itself to its host by what is known as the rostrum, in the centre of which is a barbed dart, furnished on either side with several rows of teeth arranged obliquely, which enable the tick to burrow into and adhere securely to the skin. The male tick does not suck blood, and when fully grown is very much smaller than the female. Copulation occurs whilst the female tick is sucking blood. When fully gorged she withdraws her rostrum, and, falling to the ground, lays a great number of eggs, after which she shrivels up gradually and dies. The parasite is contained in the eggs. In from two to six weeks the young ticks hatch out, creep upon the cattle, and infect them with the disease, provided that the parent tick had previously fed on cattle suffering from Texas fever. It is thus that fresh cattle arriving in infected districts acquire the disease, the native cattle suffering from a mild form without evident symptoms. It is possible that southern animals are infected as calves, and continue to harbour the blood parasites. In fact, inoculation of the blood of such animals frequently produces the infection in healthy cattle. Diseased cattle can infect healthy pastures; the ticks falling from the animals and depositing their eggs on the ground, and the young ticks subsequently communicating the disease to healthy animals. The disease breaks out in forty-five to sixty days after the appearance of the infected cattle on the pasture, because this period is required before the young brood of ticks is capable of hatching

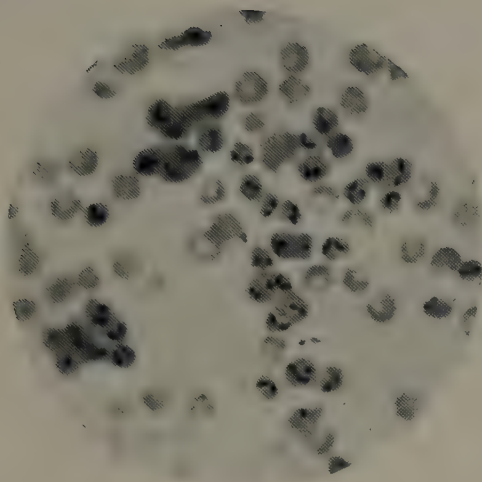


FIG. 130.—*Pyrosoma bigeminum* in blood of ox. Stained with gentian violet. $\times 900$.

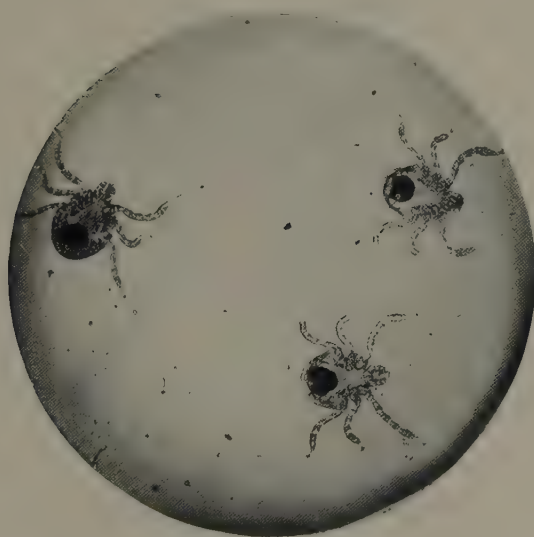


FIG. 131.—Young Ticks (*Boophilus bovis*). $\times 10$.

out and communicating the disease. The disease can also be artificially conveyed to healthy districts by dropping gravid female ticks upon the land. (See Fig. 131 of young tick).

Koch has confirmed the researches of Smith and Kilborne. He was able to infect healthy cattle with ticks taken from diseased ones. According to Smith and Kilborne, in America cattle can acquire immunity against Texas fever, but immunity is transitory unless there have been repeated attacks. The immunity conferred by a single attack, although not always preventing an attack of fever, may so mitigate the contagion as to prevent the death of the animal. Similar results were observed in Queensland.

Prevention.—Dipping the cattle to kill the ticks has been employed in America, and the best results were obtained with chloronaphtholeum, 2 per cent. (50 lbs.) with 40 lbs. of soap, in a vat 5 feet deep, and containing 2500 gallons of water. Twenty-four hours after dipping, all the smaller ticks were dead, also many gorged ones. After four to five days, all the ticks had turned black and died.

Literature of Texas Fever.—Cooper, *Journ. of Compar. Med. a. Veterin. Archives*, 1891, pp. 313-319. Smith, T., and Kilborne, F., "Investigations into the Nature, Causation, and Prevention of Texas or Southern Cattle Fever," Bulletin No. 1, Bureau of Animal Industry, U.S. Department of Agriculture, Washington, 1893 (301 pp., with 10 coloured plates). Billings, F., *Southern Cattle Fever (Texas Fever)*, Third Edition, Lincoln, Nebraska, U.S.A., 1893. Pound and Hunt, see article "Texas Fever in Australia," in Twelfth Annual Report, Bureau of Animal Industry, U.S. Department of Agriculture, 1895, pp. 85-95 (Washington, 1897). Bowhill, T., "Reports on Southern Fever in California," Fourth and Fifth Annual Reports of the Bureau of Animal Industry, U.S. Department of Agriculture, 1887-88, pp. 443-450 and 453-455 (Washington, 1889). Koch, R., *Reiseberichte, etc.*, Berlin, 1898. Williams, W., *Veterinary Journal*, 1896. Babes, *Compt. rend. de l'Acad. d. Sc.*, v., p. 115, 1888; also 1892. Celli and Santori, *Centralbl. f. Bakteriöl.*, xxi., Nos. 15 and 16.

ENDOGLOBULAR PARASITES OF THE DOG.

In 1895 Piana and Galli-Valerio discovered an intraglobular hæmatozoon in the dog, in Italy, which resembled the "*Pyrosoma bigeminum*" (Smith and Kilborne), and which they named "*Pyrosoma bigeminum*, var. *canis*." Koch has also seen it in South Africa, and Marchoux* verified its existence in Senegal in eleven dogs, and, adopting the nomenclature of Laveran, named it "*Pyroplasma canis*." None of the affected dogs exhibited any signs of jaundice, but there was a slight elevation of temperature corresponding to the period when the hæma-

* *Société de Biologie*, 27th January 1900.

tozoa were present in great numbers in the circulation. On the other hand, if in dogs previously infected, but in which the minutest microscopical examination failed to discover any parasites in the circulation, fever arose by some means, causing the reappearance of the intra-globular parasites, these dogs exhibited the phenomena described by Nicolle as occurring in oxen affected with these parasites, when a fresh infection corresponded to a fresh growth of the parasites.

In the blood of a dog suffering from malignant infectious jaundice, Leblanc* found a considerable number of hæmatozoa analogous to those found in the ox and sheep suffering from hæmoglobinuria. Leblanc† is also led to think that in every case of infectious jaundice of the dog, the spherical or ovoid parasites are analogous to those found by Marchoux in Senegal. Laveran's method of staining the parasite is stated to give the best results.

MALARIAL PARASITES.

The parasites causing malaria in man were discovered by Laveran in 1880. Five years later, Golgi demonstrated the relation existing between the development of the parasites within the body and the periodic febrile attacks. Recent investigations by Ross, Grassi, Bignami, Bastianelli, have demonstrated that the human and avian parasites undergo development in certain species of mosquito, after these insects have sucked the blood of malarial subjects. Malarial parasites undergo two cycles of development—an *asexual* cycle (so-called "sporulation") in the bodies of warm-blooded animals, and a *sexual* cycle within mosquitoes. Though it has been proved by experiment that malaria can be communicated to healthy men by injections of malarious blood taken from affected persons, infection under natural conditions would appear to occur solely through the agency of infected mosquitoes.

The malarial parasites live and multiply in the blood corpuscles. The youngest parasites are very minute, measuring 1 to 2 μ . They penetrate the red corpuscles, and grow at the expense of the corpuscle until they have reached their full development, and escape from the remains of the corpuscle. The asexual cycle of development takes place with a definite periodicity, and the types of fever which the different species of parasites produce have consequently been styled quartan, tertian, quotidian, etc. In quartan ague, the parasites escape from the

* *Société de Biologie*, 20th January 1900.

† *Ibid.*, 17th February 1900.

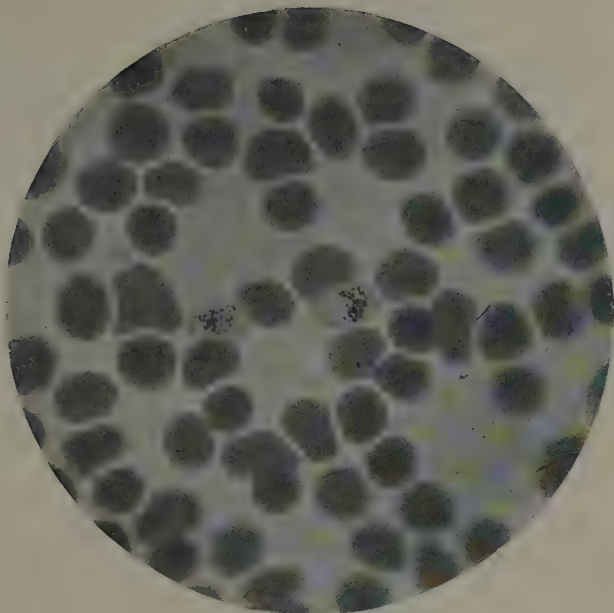


FIG. 132.—*Estivo-autumnal malarial parasites of man.* $\times 1000$.

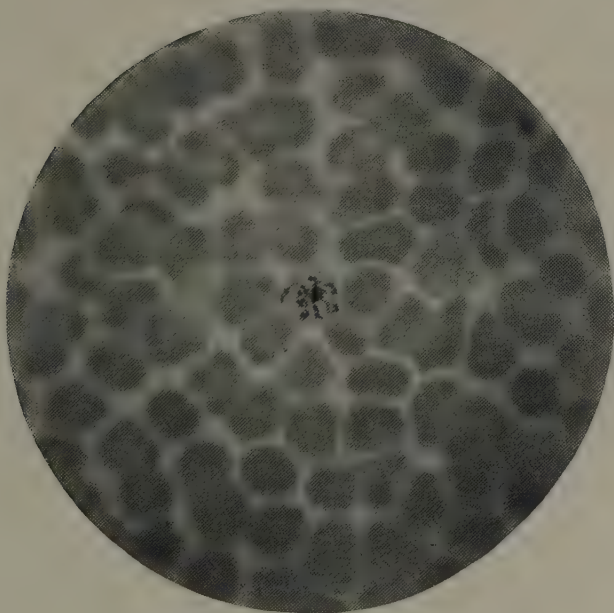


FIG. 133.—*Quartan malarial parasites of man.* $\times 1000$.

I am indebted to Dr George H. F. Nuttall, of Cambridge, for these microphotographs.

corpuscles every fourth day ; in tertian ague, every third day. An infection with two sets of parasites may produce a double tertian or "quotidian" fever. Finally, in tropical or æstivo-autumnal fever the parasites may develop irregularly, and consequently produce irregular attacks of fever. (See Figs. 132 and 133.)

In the *asexual* cycle of development the parasites, after reaching their full size (whereby they more or less fill the degenerated corpuscle), split up into a number of small spherical bodies, which are grouped in the form of a rosette. These bodies are freed with the rupture of the corpuscular wall, after which they attack fresh corpuscles, and repeat the cycle of development just described. The *febrile attack* coincides with the periodicity of this asexual cycle of development, the attack occurring at the moment when the young parasites escape into the blood-plasma and invade a fresh series of corpuscles.

The *sexual* cycle may be said to begin in man, in so far as male and female elements are developed, in the warm-blooded host. These elements develop quite independently of the attack, and instead of breaking up into rosettes, escape as large spherical bodies into the plasma, where they die off, unless taken up into the body of the insect host. If the blood of a malarial patient containing these mature forms is examined unstained under the microscope, some of them will be seen to remain quiescent, whilst others undergo so-called flagellation. Flagellation consists in the throwing-off of very actively, swimming, whip-like organs, which, breaking away, swim about in the plasma. Whereas the cells which remain quiescent are granular, the flagellating forms are hyaline. The first are the female elements, the second the male elements ; the "flagella" which they throw off corresponding, as M'Callum showed, to the spermatozoa, their function being to impregnate the quiescent female elements. In a malarial parasite affecting birds (*Halteridium*), the process of impregnation is followed by the conversion of the fertilized element into a motile, worm-like creature. What takes place under the microscope is exactly what occurs within the body of certain mosquitoes after they have sucked malarial blood containing mature parasites, only that the worm-like creature bores its way into the stomach-wall of the insect and becomes encysted in this situation. The cyst grows greatly in size, the contents breaking up into an immense number of extremely minute spindle-shaped bodies, the "sporozoites" of the Italian authors, or "blasts" of Ross. The cysts finally burst, and liberate their contents into the body-

cavity of the mosquito in which the blood is circulating. Consequently, the sporozoites are carried to all parts of the body. They accumulate, however, in the most remarkable way, in the *salivary glands* of the insect, and *escape* with the insect's salivary secretion.

The *sexual* cycle of development just described has been observed to occur in an avian parasite (*Proteosoma*) by Ross, Daniels, and Koch, as also in the various human parasites (quartan, tertian, æstivo-autumnal) by Ross, Grassi, Bignami, and Bastianelli, and other investigators.

We have used the term mosquito hitherto in a general sense as comprising culicid insects. There are two genera of *Culicidæ* which are represented by a large number of species: *Culex* and *Anopheles*. Whereas species of *Culex* have been shown to serve as hosts of the avian parasite *Proteosoma*, none of this genus have been found to fulfil this function with regard to human parasites. On the other hand, some ten or twelve species of *Anopheles* have been proved to be hosts to human parasites, and this in all parts of the world.

The development of the parasites in *Anopheles*, from the time the insect sucks blood to the appearance of the sporozoites in its saliva, usually lasts about eight days under suitable conditions of temperature. When the parasites appear in the saliva, the insect is capable of transmitting the infection in the act of biting healthy individuals. This has been proved incontestably by a number of independent experiments on man (Bignami, Bastianelli, Manson, and Rees), and birds (experiments with *Proteosoma* by Ross, Daniels, and Koch).

The investigations hitherto conducted show that some species of *Anopheles* are present wherever there is malaria. Grassi's contention that these insects are absent where there is no malaria has been disproved by Nuttall, Cobbett, and Strangeways-Pigg in Great Britain, and by Celli in Italy.

The chief differences between the human parasites are summarised in the following table :—

[TABLE

CHIEF DIFFERENCES BETWEEN HUMAN MALARIAL PARASITES.*

	TERTIAN.	QUARTAN.	ESTIVO-AUTUMNAL.
<i>Asexual cycle of development:</i> .	Takes 40 to 48 hours.	72 hours.	24 to 48 hours, irregular.
<i>Rosette forms:</i> .	Mostly in spleen and marrow, few in blood. Composed of 14 to 20 elements.	All stages found with equal frequency in blood and organs. Spores, 6 to 12.	Very rarely in blood, occur in spleen and bone marrow. Spores vary much in number, 6 to 80, or even 50.
<i>Pigment:</i> . . .	Fine, dancing, reddish-brown; tends to accumulate in bulbous pseudopodia.	Coarse, dark (usually situated peripherally) pigment; movements sluggish.	Scanty, fine; may be absent.
<i>Corpuscles:</i> . .	Tend to swell and be discoloured.	Unchanged, or smaller, coppery or greenish; some loss of colour in advanced stages.	May shrivel, be deformed and brassy.
<i>Sexually-mature forms:</i> . . .	Spherical.	Spherical.	Crescentic, becoming spherical when freed.

NAGANA, OR TSE-TSE FLY DISEASE.†

The Tse-tse Fly Disease in Africa, which is probably identical with the Indian "Surra," has been proved by Bruce (1895) to be due to a flagellated infusorian, a *Trypanosome*. By ingenious experiments, conducted in Zululand, he demonstrated that the tse-tse fly transmits the disease from diseased to healthy animals, provided that it has fed on infected blood within the preceding forty-eight hours. The disease can be transmitted by subcutaneous inoculation with a minute quantity of blood containing the parasites. It is fatal to horses, donkeys, cattle, cats, and dogs. Dogs usually die within fourteen days after inoculation, horses may live for some weeks or months, whilst the affection may last twelve to eighteen months in cattle. The number of parasites increases steadily until the animal dies. (See Figs. 134 and 135.) Bruce has calculated that the blood of a dog, prior to death, contained 140,000 parasites per cubic centimetre.

The tse-tse fly (*Glossina morsitans*) appears to act simply as a mechanical carrier, not as a host of the parasites, for these were digested within the insect, living parasites only being found in their proboscides. The disease has been produced experimentally in

* Bibliographies to the Literature on Malaria will be found by reference to Thayer and Hewetson *Johns Hopkins Hospital Reports*, vol. v.; Nuttall, *Ibid.*, vol. viii.; also *Centralbl. f. Bakteriöl.*, vols. xxv., xxvi., xxvii.; also *Journal of Hygiene*, 1901, vol. i., p. 75.

† See D. Bruce, *Tse-tse Fly Disease, or Nagana, in Zululand*, Durban, 1895; *Further Report on the Tse-tse Fly Disease, etc.*, London, 1897.

the laboratory in rats and rabbits. Bruce has shown that game (buffaloes, wildbeeste, hyena, bushbuck, etc.) can harbour the parasite.

EQUINE SYPHILIS.*

(*Fr.* Dourine.)

The *Trypanosome* causing this disease was discovered by Rouget in the blood of an Algerian horse affected with "equine syphilis." Legrain subsequently discovered the parasite in a cow in Algeria.

The disease can be reproduced by inoculation in horses, cattle, dogs, and rabbits, and may be transmitted from diseased to healthy animals in the act of *coitus*, whether the affected animal be male or female. The transmission through *coitus* has been observed in horses, dogs, and rabbits. The *Trypanosome* is present in the semen and vaginal mucus.

A horse inoculated with trypanosomal blood obtained from a jennet, showed great engorgement at the seat of inoculation, a swelling a foot in diameter developing, in the centre of which the *Trypanosomes* seemed to be confined. Numerous secondary swellings followed, the genitalia became engorged, and all the symptoms of "dourine" followed.

* See *Recueil de Méd. Vétér.*, 1896, p. 344, and 15th December 1899; *Ann. de l'Inst. Pasteur*, 1896, p. 726; *Progr. Vétér.*, 1896, p. 177; *Veter Record*, 30th December 1899.

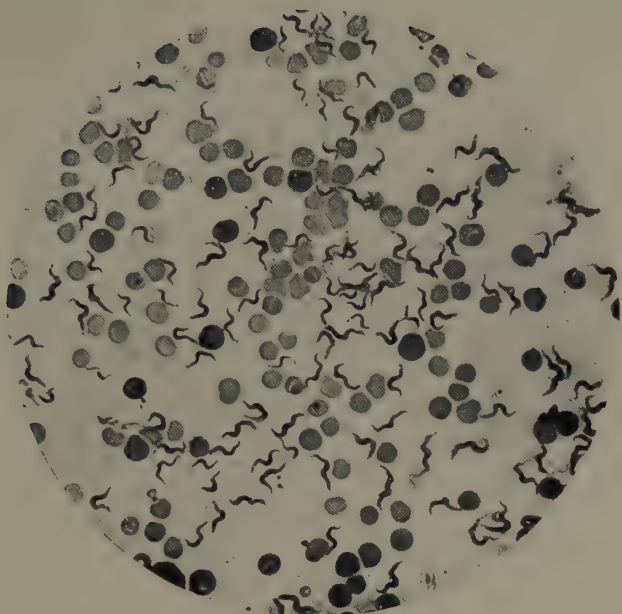


FIG. 134.—Trypanosoma of Nagana (Tse-tse Fly Disease) in blood of white rat experimentally infected. $\times 400$.

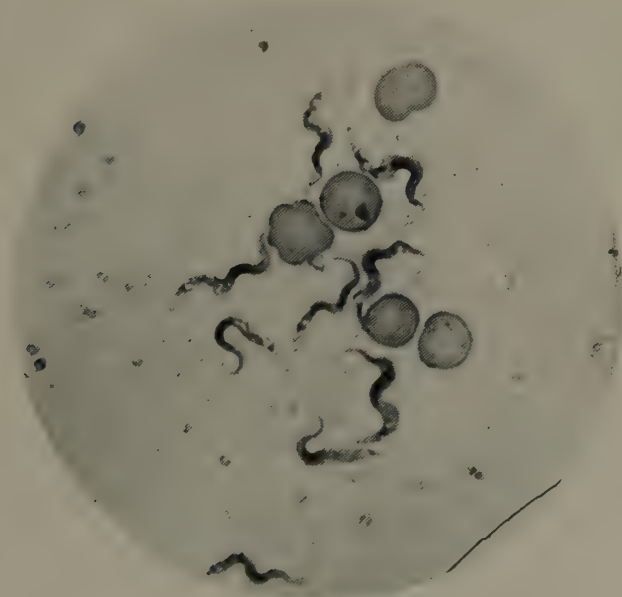


FIG. 135.—Trypanosoma of Nagana (Tse-tse Fly Disease) in blood of white rat experimentally infected. $\times 1000$.

I am indebted to Dr George H. F. Nuttall, of Cambridge, for the specimens from which I made these microphotographs.

PART VII.

Diseases due to Infective Agents of Undetermined Character.

RABIES.

RABIES has long been known as a disease occurring epidemically in dogs and wolves, which by their bites communicate the disease to man and other animals. Although the specific germ has not been isolated, we have to thank the genius of Pasteur for a successful method of treatment, which he discovered in 1885.

Animals Affected.—The disease occurs most commonly in the dog, wolf, and cat; and it may be communicated by the bites of these animals, or by inoculation, to man, cattle, horses, pigs, sheep, goats, and deer. It is stated to occur spontaneously in the skunk in the United States.

The virus is contained in the saliva of infected animals and man, but also in the central nervous system and nerve trunks, chiefly in the spinal cord. A period of incubation of variable length follows the introduction of the virus into the body. In man, the incubation period lasts usually six to eight weeks, but it may be as short as two weeks and as long as eight months. The period of incubation is shortened when the bites are situated about the face. The chances of infection are greater in proportion to the extent of the wounds inflicted by the rabid animal.

When the symptoms of the disease develop, there is absolutely no hope of recovery. The premonitory symptoms are ushered in by irritation about the bite, depression, headache, loss of appetite, sleeplessness, hypersensibility, and husky voice. The premonitory stage is succeeded by the furious stage, lasting usually one and a half to three days. This again is followed by the paralytic stage, lasting six to eighteen hours, and ending in death. In "dumb rabies" of animals only the paralytic stage may be observed.

Pasteur obtained a virus of constant virulence by passage through a series of rabbits. After the rabbits had succumbed to the disease he removed the spinal cord, and cutting it in sections, suspended these in sterilized bottles containing caustic potash or soda, which served to desiccate the cord. He found that the desiccation of the cord gradually attenuated the virus, and that by means of this attenuated virus immunity could be obtained in animals. The prevention treatment in man is usually begun with the injection of emulsions of cords dried thirteen to fourteen days, more and more virulent cords being used up to a cord dried for three days, or even less, the injections being made subcutaneously every day, or twice or thrice a day in bad cases.

About 15 per cent. of those bitten acquire rabies, and all of these would die if left untreated. The statistics of the Institut Pasteur for 1898 show only a mortality of 0.39 per cent. amongst the patients receiving antirabic treatment. To be successful, antirabic treatment should be begun early. (See p. 95 for method of inoculating a rabbit for diagnosis.)*

Diseases due to Undetermined Infective Agents which pass through Bacterial Filters.

The germs here considered cannot be classified, because of our lack of knowledge regarding them. They have all been discovered within the last four years. It will be necessary to increase the power of our microscopes in order to study them;—whether or no the modern microscope can be more perfected is still a question.

PLEURO-PNEUMONIA CONTAGIOSA BOVIS.

MM. Nocard and Roux (*Revue Vétérinaire*, May 1898) have proved this disease to be due to a very minute germ, the character of which remains to be established. The germ is so small that it passes through a Berkefeld or Chamberland "F" filter when in watery suspension, though it does not pass these filters when it is suspended in albuminous solutions. The Chamberland filter "B," which is finer than the preceding, does not permit the germ to pass, even in watery suspensions.

A trace of pleuro-pneumonic exudation was added to sterile bouillon

* A useful paper for English readers, entitled "The Shanghai Pasteur Institute," by Dr A. Stanley, has recently appeared in *The Journal of Hygiene*, 1901, vol. i., p. 260.



FIG. 186.—Contagious pleuro-pneumonia of cattle. Section of lung of cow.

in sacs of collodion, and these sacs were carefully sealed and inserted into the peritoneal cavity of rabbits. After fifteen to twenty days the sacs contained an opalescent, slightly turbid, albuminous fluid, but no germs cultivable in ordinary media. These collodion sacs permit osmosis to take place through their walls, but prevent the most minute organisms from escaping. Some rabbits in these experiments died apparently from the toxic action of the products of the germ which had multiplied within the sac, the products passing outward by osmosis through the sac wall. Microscopical examination of the contents of the sacs with a very high power (about 2000 diameters) revealed exceedingly small refractive mobile bodies, so minute that it was impossible, even after coloration, to exactly determine their form. A second sac containing bouillon, minus the pleuro-pneumonic exudation, was inserted into the peritoneal cavity of the same rabbit, in order to ascertain if the turbidity observed in the fluid of the first sac was not due simply to osmotic changes. The liquid contained in this sac was found to have remained clear. The numerous mobile bodies which, despite their extreme fineness, had rendered the inoculated liquid *opalescent*, were in reality living organisms which had undergone multiplication.

The germ was cultivated thus in the bodies of a series of rabbits, the sterile bouillon in the new collodion sac being inoculated each time from the contents of the old sac. The germ did not multiply thus in the bodies of guinea-pigs. The virus contained in the sacs taken from rabbits reproduced the disease in cows inoculated therewith. (See Fig. 136.)

FOOT-AND-MOUTH DISEASE.

(Eczema Epizootica ; *Ger.* Maul-und-Klauenseuche ;
Fr. Stomatite Aphtheuse.)

In the report of the German Commission, published February 1898, Professors Löffler and Frosch state that the many bacterial bodies heretofore described as the cause of foot-and-mouth disease do not possess the etiological significance attributed to them. The Commission were able to immunise healthy animals in various ways—calves with lymph heated for twelve hours at 37° C., and also with lymph heated thirty minutes at 60° C., and lastly, with lymph mixed with the blood of animals that had recovered from the disease—the best results being

obtained with the mixture of lymph and blood. The quantities of the mixture used were 1 to 40 c.c. of highly virulent lymph and 10 c.c. of defibrinated blood. Some of the inoculated animals were not affected, whilst others exhibited slight lesions on the mouth which did not interfere with their general health. The lesions appeared ten to fourteen days after the animals were inoculated, as flat, round, or rugged exfoliations of the epithelium, as in the natural disease. The erosions were mostly accompanied by the deposition of black or brownish pigment. The action is the same, when lymph taken from the blisters is injected into the vein on one side of the neck and the immune blood into the vein on the opposite side shortly afterwards. In different outbreaks of the disease the virulence of the lymph is extremely variable, therefore experiments are necessary to obtain, if possible, a lymph of constant action. Further experiments were also conducted with lymph diluted with 39 parts of water and mixed with a culture of the *Bacillus fluorescens* (to test if any germs passed through the filter), and the whole mixture filtered two or three times through a porcelain filter. Cultures prepared on various media from the filtrate showed no growth, thus proving that no bacteria had passed through the filter. Löffler has more recently shown that the virus is incapable of passing through a Berkefeld filter when in an albuminous fluid, though it passes readily through such a filter when suspended in watery fluids. The germ is, however, too large to pass through a Chamberland filter. A number of calves were injected intravenously with this filtrate, and at the same time others with lymph. The animals inoculated with the filtrate were affected at the same time as those inoculated with the lymph, and presented all the typical symptoms of the disease: high fever, vesicles in the mouth and feet, etc.

The Commission concluded that foot-and-mouth disease is caused by an organism of such minute size that it is able to pass through the porcelain filter. The smallest known bacterium, the *Bacillus influenzae*, measures 0.5 to 1 μ in length. If the germ of foot-and-mouth disease were only $\frac{1}{10}$ or $\frac{1}{5}$ the size of this smallest bacterium, which is possible, they would, according to Professor Abbé of Jena, be beyond the limit of the working capabilities of our microscopes, and could not be recognised with the best oil-immersion systems.

AFRICAN HORSE-SICKNESS.*

(Dutch, Paard-ziekte).

The disease known as "Horse-Sickness" appears to be peculiar to Africa. In South Africa it has been more or less prevalent since the year 1780. Two forms of the disease are recognised—(1) "*Dunnpaard-ziekte*," the characteristic symptoms of which are usually only apparent just before death. The symptoms are—heaving at the flanks, staggering gait; the animal finally falling down, when a huge cloud of foam is ejected from the mouth and nostrils. This foam, owing to its yellow colour and the length of time the ejection retains its frothy appearance, may be said to resemble the soapy contents of a wash-tub emptied on the ground, the fluid being stained slightly yellow. (2) In "*Dikkopziekte*," the second form of the disease, the characteristic symptoms are an enormous swelling of the head, neck, and lips. A condition known as "Blputong" (blue-tongue) arises when the swelling is mostly localised in the tongue, whereby the organ acquires a bluish colour due to venous engorgement. In regions where the disease is prevalent it usually appears about the beginning of November, and continues until the following May, occurring most frequently between the end of December and the latter part of February. The mortality is extremely heavy in Rhodesia and the low-lying portions of the Transvaal, where it recurs annually. In Rhodesia the annual losses amount to 90 per cent. Sporadic cases occur every year in certain parts of Cape Colony, and in some years it prevails as an epizootic, the mortality being enormous.

Nocard, and also M'Fadyean, have shown that the virus of horse-sickness passes through the pores of a Berkefeld filter but not through a Chamberland "F" filter, even when the germ is suspended in water. This proves that it is smaller than the germ of foot-and-mouth disease or of pleuro-pneumonia contagiosa (see above). Like the organism of foot-and-mouth disease, it cannot be recognised with the best oil-immersion systems.

Animals Affected.—Horses, asses, mules, and quaggas are susceptible.

* See M'Fadyean, *Journ. Compar. Pathol. and Therap.*, 1900; Report of the Director of the Colonial Bacteriological Institute for the year 1899; Nocard, 1901, *Rec. de Méd. Vétér.*, vol. viii., p. 37.

Mode of Infection.—Horse-sickness can be experimentally transmitted to healthy animals by means of the fresh blood of animals suffering from the disease, inoculation being made intravenously, subcutaneously, by means of a seton introduced beneath the skin, or by introducing the infective material into the stomach. The disease is not transmitted from horse to horse under natural conditions (Edington, M'Fadyean).

The comparative rareness of outbreaks amongst stabled horses suggests that the disease is possibly transmitted by the bites of insects. The onset of frost usually terminates the seasonal ravages of the disease.

Duration of the Illness.—Edington considers that twelve days usually elapse between infection and death in "Dunnpaardziekte," and fourteen days in "Dikkop." In the experiments conducted by M'Fadyean death usually took place on the evening of the seventh or eighth day after infection, no difference being observed in the duration of the experimental disease produced by inoculation or infection by the mouth. Edington states, the earliest indication of disease is a marked rise of temperature on the seventh or eighth day after infection, and frequently ushered in by a rigor. M'Fadyean did not observe rigors in any of his experimental cases, but noted that the temperature commenced to rise before the seventh day, the approach of death being indicated by a fall in temperature, which towards the end may have fallen to normal or even below.

Anatomical Changes.—Horses that have died from horse-sickness present lesions which vary according to the variety of the disease. On section of the skin, a gelatinous exudate is usually observed in the subcutaneous tissue in various situations, frequently being especially evident along the course of the jugular furrow. In "Dikkop" the exudate is most pronounced in the region of the head and neck. The peritoneal cavity sometimes contains a small quantity of a clear fluid, and small ecchymoses also occur on the peritoneal surface of the abdominal wall and diaphragm. The mucosa of the large intestine is sometimes coloured a livid port-wine tint, and the mucosa of the small intestine invariably exhibits a distinct inflammatory hyperæmia, sometimes being studded here and there with small petechial hæmorrhages. The liver is sometimes congested. The spleen may be normal, but it is more often slightly enlarged and congested. On section, the pulp is

observed to be very dark-coloured, and may be either soft or firm. The mucosa of the right half of the stomach is inflamed, appearing either dark-red or of a port-wine colour. In some cases the stomach shows petechial hæmorrhages. M'Fadyean reports a case in which he observed well-defined shallow erosions, somewhat larger than the surface of a split pea.

The pericardial sac is usually distended with an enormous quantity of bright yellow fluid, which in some cases is slightly or deeply tinged with blood. Edington states that he removed 140 fluid ounces from the pericardium of one case, but that the quantity present is usually under 100 ounces. M'Fadyean in his experiments never found more than a pint, and in most instances only a few ounces. In some cases the yellow gelatinous exudate extends from the base of the heart along the course of the great vessels. There is usually a considerable quantity of a straw-coloured exudate into the pleural cavity. The lungs are nearly always œdematous, and the trachea and bronchi sometimes contain a large quantity of froth. Hepatization is rare, and even the densest portions of lung float in water (M'Fadyean). The interlobular tissue in such cases is infiltrated with the clear exudate.

Diagnosis.—Edington considers that the remarkable exudation into the subcutaneous and subserous tissues is the most characteristic lesion of horse-sickness. M'Fadyean, on the other hand, states that exudations into the pleural and pericardial regions, into the subcutaneous tissues, together with œdema of the lungs, are of common occurrence, but that these appearances are not so constant as the peculiar condition of the right half of the stomach described above.

The writer, whilst Veterinary Officer with the 14th King's Hussars in the Boer War (1899-1901), had occasion to observe and make autopsies in several cases of horse-sickness amongst horses and mules. He found the gastric lesions described by M'Fadyean to be present to a greater or less degree in all cases, whereas in many cases the exudate into the pleuræ and pericardium was very variable in quantity, although always present.

The gastric lesions in horse-sickness, described so minutely by M'Fadyean, are somewhat analogous to those found on the mucosa of the abomasum in typical cases of Texas or Southern cattle fever. (See p. 292.) Horse-sickness is considered by M'Fadyean to be a

septicæmic affection, death being due to the toxic products of the germ multiplying in the blood.

Immunity.—Animals recovering from an attack of horse-sickness are spoken of as “salted,” and are believed to be protected against future attacks of the disease. It is, however, stated that animals “salted” in one district have actually died of the disease on removal to more healthy localities.

Edington states that “salted” animals are subject to attacks of irregular secondary fever, the febrile attacks varying in degree and continuing from one to six days.

Protective Inoculation.—Edington has recently described a method whereby each animal requires to be inoculated four times, at stated intervals, as follows:—A definite amount of virulent blood is mixed with 50 c.c. of standardised serum (taken from animals recovered from the disease, these animals being re-inoculated at intervals with increasing doses of virulent blood, and eight days being allowed to elapse after the last injection before bleeding the animal), and injected subcutaneously. Some days later, 30 c.c. of the same serum is injected with the same dose of blood. The procedure is repeated at a later date with a reduced dose of serum, and fourteen days later pure virulent blood is injected.

The “definite amount” of virulent blood to be mixed with the serum is not stated, and for this reason the treatment remains more or less proprietary. The results Edington claims will require to be controlled by other observers.

Prevention.—During the horse-sickness season colonists consider it inadvisable to allow animals to graze after sundown, nor before the sun has dried up the dew on the herbage. When this precaution is neglected, horses commonly sicken. Stabled horses are usually not attacked; nevertheless, in 1900, 60 per cent. of stabled horses at Echowe, Zululand, died of the sickness. The disease in this case was attributed to the animals having been fed on grass cut in the evening, and not made into bundles until the next day, as horses fed on grass previously dried in the sun were not affected.

The Boers take advantage of the fact that elevated table-lands are usually free from the disease, and send their horses to these regions previous to the onset of the horse-sickness season.

DIFFERENTIAL DIAGNOSIS TABLE.

	HORSE-SICKNESS.	ANTHRAX.
<i>Blood:</i>	Forms a clot almost normal in firmness.	Remains semi-fluid and dark-coloured.
<i>Lymph-glands:</i> . . .	Normal.	Swollen, infiltrated, and friable.
<i>Spleen:</i>	Not much enlarged; softening of the pulp limited.	Greatly enlarged; pulp usually entirely disintegrated.
<i>Microscopical examination:</i>	Negative.	Typical bacilli present and easily recognised, except when putrefaction is too far advanced.

RINDERPEST.

The specific cause of this highly infectious bovine scourge has not yet been discovered.

In natural outbreaks, about 85 per cent. of the animals attacked die. Acquired naturally, the incubative period is stated to be from four to five days; with inoculations of virulent blood that has been passed through a series of animals, usually sixty hours; and with very virulent blood, forty-eight hours. When the smallest possible quantity of virulent blood (about $\frac{1}{1000}$ c.c.) is used, the incubative period is thirteen days.

The primary symptoms of the disease are: elevation of temperature (105° F., sometimes 107° F.), disposition to lie down, rumination suspended, hair rough, nose dry, etc. About the fourth day the conjunctiva of the eye becomes reddened, associated with lachrymation, and a ropy, nasal discharge, which becomes purulent as the disease advances. Diarrhœa also occurs, associated with a peculiar tremor of the muscles of the hind-quarters, and a very diagnostic emphysema of the subcutaneous tissue. The alvine discharges become very offensive, and are often tinged with blood. About the seventh day of the fever the animal appears very distressed, breathing heavily, and getting up and lying down again very frequently. At this stage a sudden fall of temperature is generally an indication of approaching death. In some cases the temperature may fall gradually, being succeeded by a secondary fever, due to intestinal lesions and septic infection, against which the animal may struggle for a lengthened period, ultimate recovery being of rare occurrence.

In South African Rinderpest,* Koch observed that the exanthema and diphtheritic-like changes of the mucous membrane of the mouth and the palate, so common in the last English epidemic, were but little marked, whilst the other pathological lesions in the intestines are rather considerable; skin eruptions were absent.

Anatomical Changes.—The lesions observed at autopsy vary according to the period of the disease during which death occurred. When the animal dies during the primary fever, the lungs are usually normal; but when death occurs during the secondary fever, they are emphysematous.

The Heart.—Small; subserous extravasations of blood and petechiæ are often present between the ventricles, or between the auricles and the ventricles.

The Abomasum.—The mucosa is œdematous, highly congested, and covered with red petechial spots denuded of epithelium; small ulcers are also sometimes present.

The Intestines.—The intestinal tract is inflamed throughout from the duodenum to the rectum, the lesions being most marked posterior to the entrance of the bile duct, and about two feet posterior to the ileo-cæcal valve. Peyer's patches are thickened, denuded of epithelium, and studded with red ecchymosed spots, sometimes ulcerated, a greyish slough adhering to them. A complete cast of the intestine, composed of fibrin and denuded epithelial cells, is sometimes observed within the intestine. The mesenteric glands are always enlarged. *The Liver*, except in cases of secondary infection, is normal; but the gall bladder is usually inflamed, the mucosa hypertrophied, and studded with minute hæmorrhagic spots. The surface of the urinary bladder presents a similar appearance; the lesions are, however, not so pronounced, and in the majority of cases the colour of the urine is normal. The spleen and kidneys in uncomplicated cases present no special lesions. The nasal mucosa is inflamed, and of a dark plum colour.

Immunity.—Koch experimented with fowls, doves, pigeons, guinea-fowls, and a crane, with negative results.† Kohlstock inoculated a male and a female camel, with negative results, and

* R. Koch, "Report of his Investigations into Rinderpest," Kimberley, Dec. 1896 to March 1897, p. 9.

† Kohlstock, "Report of Rinderpest Investigations at Kimberley," May 1897, p. 7.

concludes they are immune by nature. Kohlstock also states, "that post-mortem lesions reveal the fact that foetal calves suffer in utero, the lesions being visible in the stomach and intestines;" and according to Turner,* "the fact appears well proved that animals in utero during the time that the mother is undergoing immunisation by gall, are born immune."

Prevention.—The following methods were adopted during the recent outbreak of Rinderpest in South Africa:—

1. Koch's original bile method.
2. Glycerinated bile method (Edington).
3. Serum method of Turner and Kolle.
4. Defibrinated blood method.

When Koch commenced his experiments, he first attempted to produce immunity with the serum of a salted animal; Watkins, Pitchford, and Theiler had, however, previous to this, produced immunity with defibrinated blood, a method further perfected by Danysz and Bordet.

Koch confined his attention to the bile method, as he considered that it promised more immediate results.† Hutcheon, in a recent report, gives a very lucid summary of the various methods and results obtained during his extensive experience with this scourge, and recommends that "pure fresh bile" should not be used in an infected herd if any of the other inoculating materials can be obtained, as it tends to intensify the character of the disease in "those already infected," and the immunising effect is too slowly developed to protect the healthy cattle against infection if they are left in contact with those already sick. "Where clean herds are in danger," they should be inoculated with large doses of glycerinated bile, from 20-30 c.c., corresponding to the size of the animal, and this inoculation followed in from eight to twelve days with an injection of a large dose (10-20 c.c.) of strong pure bile. If this method is properly carried out, such inoculated animals would have a lengthened immunity conferred on them sufficient for all practical purposes.

Hutcheon also states, in his experience with Krause's recommendation to follow the two bile inoculations with an injection of virulent

* Turner, "Rinderpest: Its Pathology, and means used to combat its invasion of South Africa," 1897, p. 13.

† D. Hutcheon, C.V.S., "Rinderpest," *Agricultural Journal*, Capetown, 8th June 1899.

blood, that "one dose" of virulent blood injected on the tenth day after the bile inoculation does not strengthen or extend the immunity conferred by the bile; if such bile possessed strong immunising properties, the blood inoculation that followed produced no febrile reaction; on the other hand, if the bile was weak in immunising properties, the mortality that followed the virulent blood inoculation was very high, in many cases 75 per cent. and even more.

With infected herds, Hutcheon recommends that they should be inoculated at once with either serum or glycerinated bile: every animal which, judging by a rise of body-temperature, would appear to be infected, should receive a large dose of not less than 100 c.c. of serum, or 30 c.c. of glycerinated bile; the latter being by preference injected into the jugular vein, so as to secure its immediate action. Then, after eight to twelve days, all the animals in a herd which are free from fever or give no indication of being infected with the disease, should receive an injection of pure bile, not less than 10 c.c., and for large animals 20 c.c. This will confer a lasting immunity sufficient for all practical purposes.

The Serum Method.—With this method of inoculation it is stated that although all the cattle in a herd are given the disease in a more or less modified form, it is comparatively rare that infection is carried from such a herd when ordinary precautions are taken.

According to the French,* the serum method only gives a temporary immunity, lasting about two months. If a longer period of protection is required, the animal must be injected with virulent blood at the same time.

All experts agree that if Rinderpest appears in a herd of cattle which have not been previously inoculated with bile, that they should be immediately inoculated with strong immunising serum if it can be obtained. According to the French, if an animal is already attacked at the time of inoculation, the serum appears to have a curative effect, and to diminish the gravity of the attack; but, on the other hand, if the animal is suffering from lachrymation, discharge, and diarrhœa, inoculation is useless, as it has no inhibitive action at this stage.

Method of obtaining Serum.—A naturally-salted animal, or one

* "Instructions for Preventive Inoculation against Rinderpest," *Revue Indo-Chinoise*, ref. *The Veterinary Record*, 22nd June 1901.

which has been given the Rinderpest after being slightly immunised, after recovery from the disease receives an injection of 100 c.c. of virulent blood, which generally produces a febrile reaction ; as soon as this reaction is past, 200 c.c. are injected, and the dose is increased to 500, 1000, 2000, 3000, and 4000 c.c. at once (5000 c.c. is stated to be the lethal dose), always waiting for the reaction from the last inoculation to subside before administering the next. From such a salted animal 30 c.c. of defibrinated blood, or its equivalent, 20 c.c. of serum, is an effective dose. According to Turner, such highly-fortified animals as would produce strong immunising serum could not be prepared in less than three months ; hence bile must of necessity be used for the inoculation of herds, or in fresh outbreaks of an isolated and sporadic character. An infection of as definite and severe character as possible can be obtained by injecting 1 c.c. of virulent blood into the jugular vein on one side of the animal, and 5, 10, 20 c.c. of serum on the opposite side. In this instance it is claimed that the organism, entering the animal by means of the virulent blood, has time to take effect before the serum has time to act. The virus is thus attenuated and not destroyed ; the animal acquires the disease in a mild form, develops all the usual symptoms, but subsequently recovers, and is salted. Hutcheon objects to this method being employed in Cape Colony, on account of the danger of introducing active Rinderpest and other diseases, such as Red Water.

Directions for preparing the Bile.—The bile should be taken from an animal in the last stages of collapse, or immediately after death. Biles of all shades of colour may be used, except these coloured red from the presence of blood, as long as they are clear and free from a putrid smell ; thin, light-yellow biles must also be rejected. All galls extracted at one time should be mixed together after standing separately for twelve to eighteen hours, so as to render them uniform in strength and immunising properties. Pure bile, unless kept in an ice-chest, must be used the second day after it is drawn. Glycerinated bile is prepared by adding one part of glycerine to two parts of bile, and allowing the mixture to stand for eight days, but in an emergency it can be used in forty-eight hours. It is injected in doses of 20 to 30 c.c., according to the size of the animal, not only with safety but with marked results.

Directions for preparing Defibrinated Blood.—The blood is

obtained from a healthy, salted animal, at least six months after it has recovered from Rinderpest. Where it is possible, such salted animals should be fortified by injections of virulent blood, commencing with 10 c.c., then 20 to 50, and lastly 100 c.c., at intervals of ten days.

Take a clean enamelled or tin pitcher, with a lid, and wash it thoroughly; fill it with water, and boil the water in it for fifteen minutes.

Cast the salted animal, cut away the hair over the jugular vein, wash the skin thoroughly, and disinfect with a 5 per cent. solution of carbolic acid, or sheep dip, in water. Empty the water out of the bucket, and bleed the animal into it, and at the same time whip the blood with an iron brush until it is covered with the white fibrin of the blood; this process takes from ten to fifteen minutes. Strain the defibrinated blood through a well-boiled piece of muslin or butter cloth.

The dose is 100 to 200 c.c., injected under the skin of the sick animals—not into the muscles—either behind the shoulder or into the dewlap. In place of whipping the blood with an iron brush, the French recommend the addition of a sufficient quantity of a concentrated sterilized solution of oxalate of soda, which prevents the blood coagulating.

INDEX.

- ABBÉ condenser, the, 16.
 Abbot's method of describing an organism, 107.
 Abscesses, causes of, 110.
 Achorion schönleinii, 272.
 Acid-resisting bacteria, 173; method of demonstrating, 180; tubercle-like bacilli in butter, 180.
 Acne contagiosa of horses, 128; man, 129.
 Actinomyces bovis, 130; hominis, 134; musculorum suis, 133; staining sections of, 48.
 Aerobic bacteria, 2; blood, 67; gelatine, 68; glycerine, 67; grape-sugar, 67; rapid preparation of, 66; Wurtz's lactose litmus, 67.
 Agar-agar media, ordinary, 66; "slants," 74.
 Agents, differentiating, 57.
 Air, bacteria in, 99, 243; examination of, ordinary method, 99; Hesse's method, 99; Petri's method, 100; Sedgewick-Tucker method, 100.
 * Amphitricha, 5.
 Amoeba coli, 290.
 Anaerobic culture methods, 80; Buchner's, 82; Fraenkel's, 80; Hesse's, 80; Kasperek's, 82; Koch's, 77, 80; Liborius's, 80; Votteler's, 83.
 Anilin dyes, 14, 50-56; oil, 51; water, 51.
 Animals, autopsies on, 97; inoculation of, 92.
 Anthrax, bacillus of, 5, 33, 139; antidotal effect of *B. pyocyaneus* in, 126; bacteriological diagnosis of, 143; differential diagnosis of, 144; differential diagnosis table, 155; in man, 141; inoculation into animals, 142; involution forms, 140; Nuttall's insect experiments with, 20, 143; symptomatici, 145.
 Anti-tetanic serum, 153.
 Anti-toxin, 203.
 Arnold's steam sterilizer (Bowhill's modified), 9.
 Ascococci, characters of, 4; billrothii, 262.
 Asiatic cholera, bacillus of, 191; bacteriological diagnosis of, 195; bacillus of, examination of water for, 196; specific reactions with bacillus, 193.
 Aspergilli, characters of, 276; in pseudo-tuberculosis, 188.
 Aspergillus, albus, 276; clavatus, 276; flavescens or flavus, 276; fumigatus, 277; glaucus, 277; nidulans, 276; niger, 276; ochraceus, 276; oryzae, 276; repens, 276; subfuscus, 276.
 Australian tick fever, 295.
 Autoclave, the, 11.
 Avian tuberculosis, 185.
 BACILLI, general characters of, 3, 4.
 Bacillus, acidi lactici, 254; acid-resisting, tubercle-like, 173; aceticus (Hansen), 258; acne contagiosa of the horse, 128; aërogenes capsulatus, 241; anaërobius, II., III., IV. (Flügge), 253; anthracis, 5, 20, 33, 139; anthracis symptomatici, 145; aquatilis, 246; arborescens, 245; argenteo-phosphorescens, 263; auranticus, 243; botulinus, 155; bovisepiticus, 215; broncho-pneumonia bovis, 237; brunneus, 244; bubonic plague, 233; buccalis maximus, 242; butyricus, 259; canary septicaemia, 221; capsulatus, 264; cholerae asiaticæ, 191; cholerae columbarum, 217; cholera of chickens, 218; cholera of ducks, 217; chromogenic, 245; coli communis, 210; coprogenes foetidus, 232; cyanogenus, 254; diphtheriæ, 199; diphtheriæ columbarum, 204; diphtheriæ vitulorum, 205; dysenteriae, 216; endocarditis

- verucosa, 232; enteritidis, 251; equi intestinalis, 212; erysipelatis suis, 230; felis septicus, 223; figurans, 161; fuscus, 244; gastratomycolosis ovis, 149; glanders, 47, 163-168; glischrogenus, 243; grouse disease, 220; gumosus, 258; helvolus, 245; hog cholera, 224; indicus, 263; indigenus, 264; influenza, 212; influenza, pseudo-, 215; janthinus, 245; lacticus, 255; lactis acidi, 255; lactis albus, 256; lactis "bleischii," 256; lactis erythrogenes, 257; lactis "Flügge," 256; lactis inocuus, 256; lactis pituitosi, 257; lepræ, 189; limbatus acidi lactici, 257; liquefaciens, 246; liquefaciens bovis, 238; liquidus, 247; malignant œdema, 144; mallei, 163; megaterium, 265; mesentericus, 246; morificans bovis, 160; non-liquefaciens, 244; œdematis maligni, 144; mouse septicæmia, 223; murisepticus, 223; mycoides, 246; neapolitanus, 169; orchiticus, 168; pasteurianus, 259; petersii, 259; phasiani septicus, 219; phosphorescens, 263; pneumonia ("Friedländer"), 118; pneumonia of turkey, 221; pneumo-enteritis of sheep, 222; prodigiosus, 210; proteus fluorescens, 162; proteus mirabilis, 160-162; proteus resembling typhoid, 244; proteus vulgaris, 160; proteus zenkeri, 160-163; pseudo-tuberculosis, 187-190; pyocyaneus, 125; radicolica, 268; rouget, 230; rubefaciens, 244; rubidus, 245; septicæmiæ hæmorrhagicæ group, 215; septic pleuro of calves, 216; smegmatis, 172, 190; spinosus, 247; subflavus, 244; subtilis, 247; suisæpticus, 229; swine erysipelas, 230; swine fever, 224; swine plague, 224; symptomatic anthrax, 145; syphilis, 173; tetani, 150; tuberculosis, 171; tuberculosis avian, 185; tuberigenus, III. and V., 269; typhi abdominalis, 104, 205; typhi murium, 223; typhoid, 205; urææ, 243; violaceus, 245; viscosus, 245; viscosus cerevisiæ, 260; viscosus lactis, 258; viscosus sacchari, 261; viscosus vini, 261.
- Bacteria, acid-resisting, 173; acidi lactici, 257, 258; aërobic, 2; aëro-anaërobic, 3; air, 99, 243; anaërobic, 2; causing acetic acid fermentation, 258; causing butyric acid fermentation, 259; causing ropiness in milk, 258; causing specific changes in beer, urine, and sugar, 260; chromogenic, 2, 243; classification of, 3; culture methods, 72; denitrifying, 2; dimensions of, 5; effect of light on, 3; effect of temperature, 3; facultative, 3; filters, 91; in inflammation and suppuration, 109; involution forms of, 5; in leguminous nodules, 268; in meat-poisoning, 155-168; in milk, 252; in urine, 243; in water, 102, 243; morphology of, 3; motile organs of flagella of, 5; mouth, 242; multiplication of, 4; nephritis, 118; nitrifying characters of, 2; nitrifying, 270; not successfully cultivated artificially, 242; photogenic, definition of, 2; points to be observed in describing, 107; pyrogenic, definition of, 2; saprogenic, definition of, 2; soil, 105, 243; special reactions during growth, 88-91; staining by Claudius' method, 21; staining by Czapelewski's method, 19; staining by Gram's method, 20; staining by Kischensky's method, 19; staining by Semenovitch and Marzinowsky's method, 19; staining capsules of, 28; staining in sections, 40; thermophilic characters, 3; thiogenic, definition of, 2; zymogenic, definition of, 2, 37.
- Bacteriological investigation, general methods of, 15-108; reagents for daily use, 14, 50; stains for daily use, 50; working bench accessories, 13.
- Bacterium, acidi lactici, 254; "Peter's," 259; phosphorescens, 263; "zopfii," 265.
- Balantidium coli, 291.
- Barbone dei bufali, 215.
- Beggiatoa, 267.
- Billing's swine fever, 224; immunity in, 228; corn-stalk disease, 237.
- Black-quarter, 20, 145.
- Blastomycetes, I, 281, 289.
- Blenorrhœa neonatorum, 124.
- Blood, examination of, 18, 34.
- Blood-serum media, fluid, 68; serum, Löffler's method of preparing, 69; serum, Nuttall's method of preparing, 69.
- "Blutputong," or blue-tongue, 305.
- Booker's roll-culture method, 75.
- Boophilus bovis, 294.
- Botkin's apparatus for anaërobic plate-cultures, 81.

- Botryococcus ascoformans*, 127.
Botriomyces, 127.
Botulinus, 155-158; bacteriological diagnosis of, 158; immunity in, 158.
 Bouillon, preparation of, 59; carbol, 60; glycerine, 60; grape and milk sugar, 60.
 Bovine farcy, 136.
Bovis, bacillus in moribificans, 160; tuberculosis, 171; tuberculosis in meat and milk, 177.
 Bowhill's stain for flagella and bacteria, 30, 53; steam sterilizer, 9.
 Bradshot, 149.
 Braxy, 149.
 Brustseuche der pferde, 126.
 Broncho-pneumonia bovis, 237.
 Bubonic plague, bacillus of, 233; vitality of, 234; Nuttall's experiments with, 235; bacteriological diagnosis of, 236; immunity in, 237; Haffkine's serum, 234.
 Buchner's method for anaërobic cultures, 82; tube for anaërobic cultures, 82.
 Bunge's stain for flagella, 32; mordant, 54.
 Busse's method of staining yeast-cell sections, 48.
 Butter, acid-resisting bacteria in, 180; bacillus tuberculosis in, 179; Roth's method of examining for bacteria in, 26.
 CANARY-BIRD, septicæmia of, 221.
 Canine distemper, 238; tuberculosis, 182.
 Capsules of anthrax, Kern's method in cultures, 29.
 Capsules, bacteria, 5; Friedländer's, 28; John's, 28; Kern's, 29; methods of staining, 28; Nicolle's, 29; Ribbert's, 29.
 Catarrh, 118.
 Catarrhal mastitis, 121.
 Cattle fever, 292.
 Cats, bacillus septicus in, 223.
 Charbon, 139; symptomatique, 145.
 Chicken, cholera, 218; disease, proteus fluorescens in, 162.
 Chlorophyll, absence of, 2.
 Cholerae asiaticæ, 191.
 Cholera, of chickens (des poules), 218; ducks, 217; hog, 224; infantum, 162; of pigeons, 217.
 Chromogenic, definition of, 2.
 Cilia, 3.
 Claudius stains, for cover-glass specimens, 21; sections, 44.
 Clathodrices, general characters of, 5.
 Cladothrix dichotoma, 266; intricata, 267; ochracea, 267.
 Classification of bacteria, 3.
 Cleansing solution for glasswork, 56.
 Clostridium, 6; butyricum, 259.
 Contrast-stains, 54.
 Cocci, general characters of, 4.
 Coccidia, 291; cattle, 291; in lambs and pheasants, 291.
 Coccidium oviforme, 291; perforans, 292.
 Colon bacillus, 210; Cesaris - Demel's differentiation method, 211; differential diagnosis table, 212; Piorkowski's differentiation method, 211.
 Comma bacillus of Koch, 191.
 Contact specimens, preparation of, 18.
 Corn-stalk disease, 237.
 Cover-glass specimens, Claudius method of staining, 21; contact method of staining, 18; Czapelewski's method of staining, 19; examination of, 18; glasses used, cleansing of, 57; Gram method of staining, 20; Kischensky's quick method of staining, 19; Löffler's method for cleansing, 56; method of remounting and retaining, 36; necessary precautions with, 36; preparation of, 17; Semenowicz and Marzinowsky's quick method of staining, 19; smear method of staining, 17; Zettinow's method for cleansing, 56.
 Cryptococcus of Rivolta, 169.
 Culture media, preparation of, 59.
 Cultures, method of detecting indol and nitrates in, 90; method of maintaining pure, 79; method of obtaining pure, from plates, 79; method of preparation of, from organs, tissues, etc., 98.
 Czapelewski's method of staining bacteria, 19.
 Czinziński's solution, 53.
 DARK closets for culture, necessity of, 3.
 Demel, Cæsaris-, differentiation of colon bacillus, 211.
 Denecke's vibrio, 197.
 Dermatomyces gallinarum, 273.
 Differentiating reagents, 57.
 Dikkopziekte, 305, 306.
 Dimensions of bacteria, 5.
 Diphtheria, anti-toxin, preparation of, 203; anti-toxin, Schering's, 204; bacillus of, 24, 199; differential diagnosis of,

- 202; experiments of Schottelius, 201; immunity, 202; of calves, 205; of chickens, 204; of pigeons, 204; Roux's method of immunizing horses, 203; staining method of Neisser, 24.
- Diphtheritic membrane, examination of, 107.
- Diplococci, general characters of, 4.
- Diplococcus, intracellularis, 115; of pneumonia, 113; of pleuro-pneumonia in horses, 126.
- Discomyces, 127.
- Disinfecting solutions, 58.
- Dog, endoglobular parasites of, 295.
- Drumstick bacteria, 6.
- Druse der pferde, 119.
- Dry heat sterilization, 7.
- Duck cholera, 217.
- Dunbar's fermentation tube, 89.
- Dunham's peptone media, 62.
- Dunpaardziekte, 305, 306.
- Dysenteria, hæmorrhagica coccidiosa, 291; vitulorum, 216.
- EARTH, examination of, 105.
- Eczema epizootica, 303.
- Egg media, 64; albumen, 64; Günther's method, 64; Hueppe's method, 64.
- Ehrlich's anilin-water stains, 51; stain for tubercle bacilli in cover-glass specimens, 23; stain for tubercle bacilli in cover-glass sections, 46.
- Elsner's method for demonstrating typhoid bacilli, 104.
- Embedding in celloidin, method of, 40.
- Endocarditis verucosa bacillosa, 232.
- Endospore formation, 6.
- Enteritidis, bacillus of, 158, 251; symptoms in man, 159.
- Equi intestinalis, bacillus of, 212.
- Equine syphilis, 300.
- Equine tuberculosis, 181; abdominal form, 181; distinctive features of, 181; thoracic, 181.
- Erysipelas, streptococcus of, 112.
- Esmarch's apparatus for counting colonies, 78; method for potato media, 61; roll-culture method, 74.
- Excelsior gas valve, 85.
- "FACULTATIVE" organisms, 3.
- Fæces, examination of, for tubercle bacilli, 27.
- Farcy, African, 169; in cattle, 136.
- Favus, 70.
- Fermentation, production of, 88; tube, Dunbar's, 89.
- Finkler's and Prior's vibrio, 196.
- Fisher's stain for flagella, 32.
- Flagella, 5, 29; Bowhill's method of staining, 30; Bunge's method of staining, 32; Fisher's method of staining, 32; Löffler's method of staining, 29; Pitfield's method of staining, 31; Van Ermengem's method of staining, 31.
- Foot-and-mouth disease, 303.
- Foulerton's medium for gonococcus, 70.
- Fowl cholera, 218; immunity in, 219.
- Fraenkel's anaërobic culture method, 80; diplococcus of pneumonia, 113.
- Freezing, preparation of tissues for, 37.
- Friedländer's bacillus of pneumonia, 118; stain for capsules of bacteria, 28, 48.
- Fungi, examination of unstained, 25; media for, 70; media, Sabouraud's medium, 70; Unna's method of staining, 25.
- Fusisporium moschatum, 278.
- Futcher's stains for malarial parasites, 35.
- GABBET'S stain for tubercle bacilli, 22.
- Gas in cultures, nature of, 90; explosive nature of, 90.
- Gastromycosis ovis, 149.
- Gelatine media, 64.
- Glanders, bacillus of, 47, 163; bacteriological diagnosis of, 166; differential diagnosis table, 171; mallein, 166; Hunting on, 166; Nocard on, 165, 167; mode of infection, 165; staining of, 23, 45, 47, 163; Straus's method for bacteriological diagnosis of, 166.
- Glassware, method of cleansing, 56.
- Glycerine media, 60, 67.
- Gonococcus, 123; bacteriological diagnosis of, 124; Foulerton's media for, 70; Knaack's stain for, 24; Neisser's stain for, 24.
- Gonorrhœa, 123.
- Gourme, 119.
- Gram's method, bacteria stained by, 21; control test, 20; Nicolle's control test, 20, 44; mordant control test, 54.
- Green pus, bacillus of, 125.
- Grouse disease, 220.

- Gruber's reaction, in Asiatic cholera, 193 ;
in typhoid fever, 208.
- "Günther's Gram" modification, 43 ; egg
medium, 64.
- HAFFKINE, anti-plague serum, 234.
- Hand centrifuge, 26.
- Hanging-drop, preparation of, 15 ; method
of examining, 16 ; Nuttall's method of
examining, 15.
- Hay bacillus, 247.
- Hearson's thermostat, 84-86.
- Herpes tonsurans, 273.
- Hesse's method for anaërobic cultures, 80 ;
method for air examination, 99 ; method
for apparatus for air examination, 99.
- Holz's potato gelatine, 61, 208.
- Horses, contagious acne of, 128 ; pleuro-
pneumonia of, 126 ; sickness, African,
305-308.
- Hot-air oven, description of, 7.
- Hydrogen in anaërobic cultures, 80.
- Hydrophobia, 301 ; inoculation of rabbits
for diagnosis of, 95.
- Hyphomycetes, general characters of, 1 ;
or mould fungi, 272.
- ICE-CREAM, examination of, for bacteria,
106.
- Icterus, 162.
- Immunity, in botulinus, 158 ; in bubonic
plague, 237 ; in diphtheria, 202 ; in fowl
cholera, 219 ; in influenza, 215 ; in ma-
lignant œdema, 145 ; in southern fever
of cattle, 295 ; in swine erysipelas, 230 ;
in swine fever, 224.
- Incubator, description of, 83.
- Indol in cultures, 90 ; methods of detecting,
90 ; reaction in cholera cultures, 193.
- Infectious broncho-pneumonia, 237.
- Inflammation, bacteria found in, 109.
- Influenza, bacillus of, 212 ; immunity in,
215 ; Pfeiffer's method for pure cultures
of, 213.
- Inoculation of animals, 92 ; of media, 71 ;
of rabbits for diagnosis of rabies, 95.
- Involution forms of bacteria, 5.
- Iodococcus vaginatus, 242.
- Ixodes bovis, 294.
- Ixodic anæmia in cattle, 292.
- JOHNE'S method of staining capsule bacteria,
28.
- Johnston, Wyatt, modification of Widal's
reaction, 209.
- Jung's students' microtome, 38.
- KASPAREK'S anaërobic culture method, 82.
- Kern's capsules of anthrax bacilli in cul-
tures, 29.
- Kipps' hydrogen apparatus, 82.
- Kischensky's method of staining bacteria,
19.
- Kitasato's anaërobic culture flask, 81.
- Klebs-Löffler bacillus of diphtheria, 199.
- Klein's method of staining spores, 34.
- Klossia soror, 292.
- Knaack's method of staining gonococci, 24.
- Koch's anaërobic culture method, 72 ;
apparatus for blood-serum, 68 ; apparatus
for plate-cultures, 72 ; method for
diagnosis of cholera bacilli, 103 ;
peptone-water solution, 62 ; plate-culture
method, 72 ; safety burner, 83 ; steam
sterilizer, 7 ; tuberculin, "new," 183 ;
tuberculin, "original," 183.
- Kühne's methylene-blue stain, 52 ; method
for sections, 42.
- LAUTENSCHLAGER'S steam sterilizer, 9.
- Leprosy, bacillus of, 189 ; Baumgarten's
method of staining, 173.
- Leptothrix, definition of, 5 ; buccalis inno-
minata, 242 ; gigantea, 243 ; maxima,
242 ; maximus, 242.
- Leuconostoc mesenteroides, 261 ; indicum,
262.
- Liboriosis' method for anaërobic cultures,
80 ; tube for anaërobic cultures, 80.
- Litmus tincture, 88.
- Lockjaw, 150.
- Löffler's, blood-serum for diphtheria, 69 ;
method for cleansing cover-glasses, 56 ;
method of staining flagella, 29 ; method
of staining glanders bacilli, 23, 47 ;
methylene-blue stain, 51 ; mordant, 54 ;
universal staining method, 42.
- Lophotricha, 5.
- Lorenz's vaccine for rouget du porc, 232.
- Lustgarten's bacillus of syphilis, 173.
- Lymphangitis, epizootic, 169 ; ulcerative,
170.
- MADURA-FOOT disease, 135.
- Malarial parasites, 35, 52, 296 ; Fletcher's
stain for, 35 ; Plehn's stains for, 36, 52,

- 53; Romanowsky's stain for, 36; stains for, 35.
- Malassez and Vinal's blue stain for pseudo-tuberculosis, 187.
- Malignant oedema, bacillus of, 22, 144; differential diagnosis table, 155; immunity in, 145; in man, 145.
- Malignant pustule, 142.
- Mallein, diagnosis of glanders' with, 166; dose of, 166; Hamilton on, 167; Hunting on, 166; Nocard on, 167; preparation of, 166.
- Malta fever, 116.
- Mammitis, catarrhal, 121; contagious, 120; gangrenous, in ewes, 126; tubercular, 178.
- Marzinowsky's, etc., method of staining bacteria, 19.
- Mastitis (*see* Mammitis), 121.
- Maul-und-Klauenseuche, 303.
- Meat, poisoning, bacteria of, 106, 155-163; tubercular, 177; unsound, 106, 155.
- Media, agar-agar, 66; blood-serum, 68; bouillon, 59; bread paste, 70; carbolic acid, 60, 65; clearing and mounting, 58; counting colonies of, 77; egg, 64; for mould fungi, 70; gelatine, 64; glycerine, 60; grape-sugar, 60, 65; inoculation of, 71; milk, 63; nutrient, preparation of, 59; peptone-water, Dunham's, 62; peptone-water, Koch's, 62; peptone rosolic acid, 62; potato, 62; preparation of tubes, flasks, etc., 71; Sabouraud's, 70; special, 69; Winogradsky's, 71.
- Membrane, diphtheritic, examination of, 107.
- Micrococci, general characters of, 3.
- Micrococcus, *acidi lactici*, 252; *acidi lactis liquefaciens*, 253; *agilis*, 249; *aquatilis*, 248; *auranticus*, 248; *candicans*, 248; *concentricus*, 248; *cremoides*, 248; *gonorrhoea*, 123; *intracellularis meningitidis*, 115; *lutea*, 249; *melitensis*, 116; of gangrenous mammitis in sheep, 126; *prodigiosus*, 245; *radiatus*, 249; *rosetaceus*, 248; *sarcina lutea*, 249; *tetragenus*, 122; *ureæ*, 243; *versicolor*, 248.
- Micron, a, 5.
- Microscope of Leitz, 15; of Zeiss, 15.
- Microtome, Jung, 38; Schanze's, 39.
- Miller's spirillum, 197.
- Milk, bacteria, 244, 246, 252, 254, 256, 257, 258; gelatine, 65; media, 63; method of examination of bacteria, 25; sugar bouillon, 60; tubercle bacilli in, 177.
- Milzbrandbacillus, 139.
- Moist heat sterilization, 9.
- Monas prodigiosa*, 245.
- Monotricha*, 5.
- Money's method of staining bacteria, 46.
- Morbificans bovis*, 160; action on man, 160.
- Mordants, 54.
- Morphology of bacteria, 3.
- Mould fungi, 1; media for, 71.
- Mouse septicæmia, 221.
- Mouth, bacteria found in, 242.
- Mucors, characters of *corymbifer*, *mucedo*, *pusillus*, 277; characters of *ramosus*, *racemosus*, *rhizopodiformis*, *stolonifer*, 278.
- Mycoderma aceti*, 258; *cerevisiæ et vini*, 286.
- Mykodermoid, "Johne," 127.
- NAGANA, 299.
- Nasal catarrh, 118.
- Neisser's stain, for *diphtheria bacilli*, 24, 52; for *gonococci*, 24.
- Nephritis, 118.
- Nicoll's carbol-thionin stain, 53; methylene-blue, 45; thionin method, 45; method* of staining capsules, 29, 49; tannin method, 45.
- Nitrates, 271.
- Nitrifying bacillus of Winogradsky, 270.
- Nitro-bacteria, 270.
- Nitroso-bacteria, 270; *nitrosococcus braziliensis*, 271; *nitrosomonas europæa*, 270; *nitrosomonas javaniensis*, 270.
- Nitroso-indol reaction in Asiatic cholera, 193.
- Nutrient gelatine, preparation of, 64.
- Nuttall's experiments with bacillus anthracis, 143; with bacillus of bubonic plague, 233; microscope-thermostat, 16; platinum culture spear, 98; roll-culture apparatus, 74-77; steam sterilizer, 10; sterile fluid-serum method, 69.
- OIDIUM *albicans*, 274; *lactis*, 275.
- Onychomycosis trichophytina, 273.
- Orchitis caused by bacillus mallei, 166; by bacillus orchiticus, 168; by bacillus of ulcerative lymphangitis, 170.

- Organs, etc., preparation for examination, 37.
- Organism, points to be observed in describing, 107.
- Otitis media acuta, 118, 215.
- Ovis gastromycosis, 149; pseudo-tuberculosis, 190.
- PAARD-ZIEKTE, 305-308.
- Paraffin, hardening tissues in, 39.
- Paramæcium coli, 291.
- Parasite, definition of, 2.
- Parrots, disease of, 113.
- Pasteur's anthrax vaccine, 142; chicken cholera anthrax vaccine, 219; rouget anthrax vaccine, 232.
- Pathogenic blastomycetes, 289.
- Peckham's indol reaction with bacillus typhi-abdominalis, 208.
- Penicillium glaucum, 275.
- Peptone, rosolic acid media, 62; water medium, "Dunham's," 62; water medium, "Koch's," 62.
- Peritricha, 5.
- Petri-dishes, cultures in, 73; sterilization of, 8.
- Petri's method for examination of air, 100.
- Pfeiffer's method for pure cultures of influenza, 213; method of staining bacteria, 46; reaction for bacilli of Asiatic cholera, 193.
- Pheasant disease, 219.
- Phosphorescent bacteria, 263.
- Photogenic, definition of, 2.
- Pig, pneumonia of, 229.
- Pitfield's stain for flagella, 31, 54.
- Piorkowski's differentiation method for typhoid and colon bacilli, 211.
- Plasmodia, 3.
- Plasmodium malarie, Plehn's stain for, 52; Romanowsky's stain for, 53.
- Plate-cultures, methods for hard substances, favus, etc., 71-74; methods of counting colonies in, 77; "Koch's" original method, 72; quantitative method, 79.
- Plehn's stain for malarial parasites, 36.
- Pleuro-pneumonia contagiosa, 302; No-card's investigation with, 302; experimental inoculation, 303.
- Pneumococci in pneumonic sputum, staining of, 27.
- Pneumo-enteritis of sheep, 222.
- Pneumonia, bacillus of Friedländer, 118; broncho-, infectious, bovis, 237; diplococcus, of Fränkel, 113; infectious, of pigs, 229; pleuro-, contagiosa, of cattle, 302; pleuro-, contagiosa, of horse, 126; pleuro-, septic, of calves, 216; in swine fever, 224; of turkeys, 221; post-mortem examination of inoculated animals, 97.
- Potato, bacillus, 244, 245, 246, 247; culture media, 60; culture, Esmarch's method, 61; culture, Holz's method, 61; culture, Roux and Globig's, 61; culture, water, 62.
- Precautions in staining of sections, 49.
- Proteus, fluorescens, 162; in chicken disease, 162; mirabilis, 162; vulgaris, bacillus of, 160; vulgaris, bacteriological diagnosis of, 162; vulgaris, in cholera infantum, 162; vulgaris, putrid intoxication with, 162; vulgaris, swarming "islands," 161; vulgaris, symptoms of infection in man, 162; vulgaris, zenkeri, 160, 163.
- Protozoa, 3, 290.
- Pseudo-diphtheria, bacillus of, 202; differential diagnosis, 202; influenza, bacillus of, 215.
- Pseudopodia, 3.
- Pseudo-tuberculosis, 136, 179, 187; animal parasites, cause of, 187; aspergillus glaucus and fumigatus, causes of, 188; in calves, in cats, 187; causal factors of, 187; Courmont's, 188; in dogs, 187; Malassez and Vignal's, 187; in man, 187; in pigeons, 188; in sheep, 187, 190.
- Pus, blue and green, bacillus of, 125.
- Pustula maligna, 142; pyocyanin, 125.
- Pyogenic, definition of, 2.
- Pyrosoma bigeminum, 292.
- RABIES, 301; diagnosis of, 95.
- Ragpickers' disease, 142.
- Rauschbrand bacillus, 145.
- Reagents, differentiating, 57; special actions of, 58.
- Red dysentery of cattle, 291.
- Reichel's filter for bacteria, 91.
- Relapsing fever, spirillum of, 198.
- Rhizobium leguminosarum, 269.
- Ribbert's stain for capsules of bacteria, 29.
- Rinderpest, 309.

- Rinderseuche, bacillus of, 215.
 Roll-cultures, 74.
 Romanowsky's stains for malarial parasites, 36, 53.
 Rosolic acid, 62.
 Roth's method for tubercle bacilli in butter, 26.
 Rotzbacillus, 163
 Rouget du porc, bacillus of, 230.
 Roux's double stain, 51; method of immunizing a horse, 203.
- SABOURAUD's medium for favus, 70.
 Saccharomyces, *acidi lactici*, 286; *anomalus*, 285; *apiculatus*, 285; *cerevisiæ* etc., I., 281; *ellipsoideus* I., 282; *ellipsoideus* II., 283; *exiguus*, 286; *hominis*, 287; *litogenes*, 288; *marxianus*, 286; *membranæfaciens*, 286; *neoformans*, 288; *pastorianus* I., 283; *pastorianus* II., 284; *pastorianus* III., 284; *subcutaneous tumefaciens*, 288.
 Saliva, bacteria in, 203.
 Sanarelli's experiments with bacillus typhi abdominalis, 210.
 Saprogenic, definition of, 2.
 Saprophyte, definition of, 2.
 Sarcinæ, characters of, 4; orange, 249; red, 249; white, 249; yellow, 249.
 Schanze's microtome, 39.
 Schering's diphtheria anti-toxin, 204.
 Schüller's experiments with tuberculosis, 176.
 Schizomycetes, I.
 Schottelius' experiments with diphtheria, 201.
 Schutz, diplococcus of pneumonia (horse), 126; streptococcus of strangles, 119-122; *tinea galli*, 229.
 Schweineseuche, bacillus of, 229; differential diagnosis, 233.
 Schweinepest, 224.
 Schweinerothlauf, 230.
 Schwenitz, immunity in hog cholera and Schweineseuche, 228.
 Sections, Busse's method of staining, 48; Claudius' stain for, 44; common faults in cutting of, 37-40; double staining of, 43; Ehrlich's stain for tubercle bacilli in, 46; examination of, for bacteria, 40; Friedländer's method for demonstrating bacteria in, 48; glander bacillus, method for, 47; Gram-Günther method of staining, 43; Löffler's method for glanders bacillus, 47; method for actinomycetes, 48; Money's stain for, 46; Nicolle's method, 44, 49; Nicolle's methylene-blue tannin method, 45; Nicolle's thionin method, 45; Pfeiffer's method, 46; universal method, 42; precautions, 49; staining of, for bacteria, 40; Unna's method for glanders bacillus, 47; Unna's dry method, 45; Weigert's stain for, 42.
 Sedgewick-Tucker method of examining air, 100.
 Semenowicz and Marzinowsky's method of staining bacteria, 19.
 Septicæmia hæmorrhagicæ, bacteria group of, 215.
 Sheep, pneumo-enteritis of, 222.
 Smear preparations, 17.
 Smegma bacillus, 172.
 Smith's fermentation tube, 89; method of isolating intestinal bacteria, 105.
 Soil bacteria, method of examination for, 105.
 Solid culture media, 69.
 Solution for cleansing and disinfecting, 56.
 Southern cattle fever, 292; anatomical changes in, 293; immunity in, 295; Koch's experiments with, 295; mode of infection, 294; parasite of, 293; ticks in, 294.
 Sphærococcus *acidi lactici*, 253.
 Spirilla, general characters of, 3, 4.
 Spirillum, dentium, 243; Finkler and Prior, 196; Miller's, 197; Obermeieri, 198; sputigenum, 243; tyrogenum, 197; undula, majus and minus, 251.
 Spirochæte, characters of, 4; Obermeieri, 198; dentium, 243.
 Spores, endogenous, 6; formation of, 5; methods of staining, Klein's, etc., 32-34; vitality of, 6.
 Stains, 50-56; Aron's, 52; Bowhill's, 53; Bunge's, 54; contrast, 54, 56; contrast, borax-carmin, 55; contrast, cochineal-alum, 55; contrast, Friedländer's picro-carmin, 54; contrast, hæmatin-alum, 55; contrast, Weigert's picro-carmin, 55; Czaplewski's, 53; Czinzinski's, 53; Ehrlich's anilin-water, 51; Gabbet's solution, 51; Kühne's, 52; Löffler's, 51, 54; Neisser's double, 52; Nicolle's, 53; ordinary, 40-50; Pitfield's, 54; Plehn's,

- 52; precautions to be observed, 49; Romanowsky's, 53; Roux's double, 51; stock solutions, 50; Weigert's, 55; Ziehl's, 51.
- Staphylococci, general characters of, 4.
- Staphylococcus, *cereus albus*, 110; *cereus flavus*, 110; *cereus griseus*, 110; *pyogenes albus*, 110; *pyogenes aureus*, 109; *pyogenes citreus*, 110; *pyogenes rosaceus*, 110; differential table, 110.
- Steam sterilizers, 7-12.
- Sterilization, by heat, 7, 12; Tyndall's discontinuous method, 11; of post-mortem instruments, 11.
- Sterilizer, hot-air, 7.
- Stomatite aphtheuse, 303.
- Strangles, differential diagnosis of, 120; streptococcus of, 119.
- Straus's method for the diagnosis of glands, 166.
- Streptococci, characters of, 4.
- Streptococcus, *acidi lactici*, 253; of catarrhal mastitis, 121; of erysipelas, 112; *hollandicus*, 253; of mammitis in cows, 120; *perniciosus psittacorum*, 113; *pyogenes*, 111; strangles in horse, 119.
- Streptothrix, *actinomyces bovis*, 130; *actinomyces*, differential diagnosis, 134; *capræ*, 137; characters of, 5, 129; *cuticuli*, 138; *Eppingeri*, 135; *farcinica*, 136; *Hoffmani*, 133; *Hominis*, 134; *maduræ*, 135; pseudo-tuberculosis, 188.
- Suppuration, bacteria found in, 109.
- "Swarming islands," 161, 163.
- Swine erysipelas, 230; differential diagnosis table, 233; immunity, 232; Lorenz's vaccine, 232; Pasteur's vaccine, 232; symptoms of, 230.
- Swine fever, bacillus of, 220; differential diagnosis table, 233; flagella, 225; identity of English and American, 235; immunity in, 228; lesions of, 227; pneumonia in, 227; symptoms of, 226.
- Swine plague, 229; mode of infection, 182; tuberculosis, 182.
- Symptomatic anthrax, 20, 145; characteristic lesions, 145; differential diagnosis of, 147; differential diagnosis table, 155; vaccination, 148; vaccination, French method, 148; vaccination, Kitt's method, 148.
- Syphilis, equine, 300.
- TABES mesenterica, 178.
- Technique, bacteriological, principles of, 13.
- Tetanus, bacillus of, 150; differential diagnosis table, 155; flagella, 150; immunity and cure of, with anti-tetanic serum, 153; Kitasato's method for pure cultures, 151; Kitt's method for pure cultures, 151; motility, 150; staining reactions, 150.
- Tetrads, general characters of, 4.
- Texas cattle fever, 292.
- Thermophilic bacteria, 3.
- Thermostat, Hearson's, 84; microscopic, Nuttall's, 16.
- Thiogenic, definition of, 2.
- Thrush, 274.
- Tinea galli, 273.
- Tissues, hardened sections of, 38.
- Torulæ, characters of, 287; white, rose, and black, 287.
- Tricophyton tonsurans, 273.
- Tuberculin, 182; dose of, 183; Koch's new preparation of, 183; Koch's original preparation of, 183; method of applying test, 183; precautions before and after use, 183.
- Tuberculosis, 171; avian, 185; bacilli, differential staining of, 173; bacilli, Ehrlich's stain for, 23, 46; bacilli, examination of fæces for, 27; bacilli, Horman and Morgenroth's butter experiments, 179; bacilli in butter, 179; bacilli in meat, 177; bacilli in milk, 179; bacilli, Koch's method for pure cultures, 173; bacilli, micro-chemical reactions, 172; of the udder, 178; bacilli, Petri's, 179; bacilli, Rabinowitsch's, 179; bacilli, Schüller's experiments with dead, 176; bacilli, staining reactions, 172; bacillus of, 5, 171; bacilli, Ziehl-Gabbet method of staining, 22; bovine, 176; bovine, prevention of, 178; canine, 182; equine, 181; of swine, 182; pseudo-, 187; zöögloëic, 187.
- Turkeys, disease of, 221.
- Typhi abdominalis, 205.
- Typhi murium, 223.
- Typhoid fever, bacillus of, 205; differential diagnosis table, 212; Elsner's method for detection in water, 104; flagella, 206; involution forms, 205; Sanarelli's experiments with, 210;

- specific reaction, 208; Widal and Gruber's reaction, 208; Wyatt Johnson on, 209.
- Typhoid of mice, 223.
- UDDER, tuberculosis of, 178.
- Ulcerative lymphangitis, 170.
- Unna's stain for fungi, 25; stain for glanders bacillus, 47; dry staining method for sections, 45.
- Urine, bacteria found in, 243.
- Urobacillus pasteurii, 243.
- VACCINATION, in anthrax, 142; symptomatic anthrax, 148; chicken cholera, 219; rouget cholera, 230; swine fever, 228.
- Van Ermengem's bacillus botulinus, 155.
- Van Ermengem's flagella stain, 31.
- Vibrio septique, 144.
- Vibrio, aquatilis, 249; berolinensis, 249; characters of, 4; cholerae asiaticæ, 191; cholerae asiaticæ, in water, 196; Den-ecke, 197; Dunbar, 117; gindha, 250; lissabon, 250; massauah, 250; metschnikovi, 250; phosphorescens, 250; proteus, 196; rugula, 250.
- Votteler's method for anaërobic cultures, 83.
- WATER, bacteria in, 246; Elsner's method for bacillus typhi abdominalis, 104; Koch's method for vibrio cholerae asiaticæ, 103; method of, examination, 102; method of quantitative, 103; quick method for pathogenic germs, 104; Smith's method for intestinal bacteria, 105; transportation case for analysis, 103.
- Weigert's stains, 41.
- Weill's disease, 162.
- White diarrhœa in calves, 216.
- Widal's reaction, 208.
- Winogradsky's solution, 71.
- Wolffhügel's counting apparatus, 77.
- Woodhead's experiments with bacillus pyocyaneus, 126.
- Woolsorters' disease, 142.
- Work-table, 13.
- Wundstarrkrampf, 150.
- Wurtz's lactose litmus agar, 67.
- YEAST cells, nuclei, method of staining, 34; fungi, 281; pure cultures of, 281.
- ZIEHL-GABBET, stain for tubercle bacilli, 22.
- Zoöglœa, characters of, 4.
- Zoöglœic tuberculosis, 187.
- Zymogenic, definition of, 2.

SOME CRITICAL NOTICES OF THE FIRST EDITION

"THIS work is remarkable, if not absolutely unique, alike in regard to the mass of information it contains, and the superexcellence of the original illustrations with which it is embellished. The author has long made a special study of bacteriological science, and as early as 1891 he proved the identity of the germs of swine fever in the United Kingdom with the bacteria of hog cholera in the United States of America. He has worked in the laboratory of Dr Günther, who is incontestably the most eminent bacteriologist of the present day. The illustrations with which the book is embellished are most admirably executed in colotype, from photomicrographs executed by the author by means of the Zeiss apparatus, and are works of art such as are rarely produced out of Germany. Alike in regard to the letterpress and the illustrations, the book is a monument to the transcendent ability of the author. As Mr Bowhill is not only a bacteriologist of outstanding ability, but a veterinary expert as well, he has naturally given prominence to the subject of bacteria which cause disease in human beings and farm live stock, but the bacteria of soil and milk have also received due attention at his hands. . . . Altogether this is a most remarkable work, and to the bacteriologist, the medical man, the aspirant for the Degree in Public Health, and the veterinarian, it should be not only useful in the highest degree, but absolutely indispensable."—*North British Agriculturist*.

" . . . The author writes lucidly, and with all the authority of an expert about such diseases as swine fever, swine plague, swine erysipelas, pleuropneumonia, contagiosa bovis, broncho-pneumonia bovis, grouse disease, etc. Moreover, glanders, diphtheria and tuberculosis, as they affect man and animals, are ably dealt with. . . . In the concluding portion of the book—Part vi., Protozoa—an excellent account is given of Texas cattle fever, plasmodium malarix, etc. . . . No student, or even expert, not only in veterinary but also in medical and sanitary science, can afford to be without a copy of this excellent manual."—*Nature*.

"This work is likely to be of great service to all students of germ-science, and also to those who are in any way interested in the life-histories of the microscopic living things which are fraught with so much danger to humanity. . . . We must specially note the excellent microphotographs with which the work is illustrated. These for the most part are clearly defined, and add greatly to the value of the book, while the section dealing with 'photo-micro-photography' should prove interesting to all engaged in bacteriological research."—*Glasgow Herald*.

" . . . The greater part of the book deals, like any ordinary bacteriological manual, with the various bacteria which cause disease in man and the lower animals. The attention paid to the latter is a special and useful feature in the book."—*Scotsman*.

"Mr Bowhill has justification for adding to the list of text-books on bacteriology, for his book may be said to be a handbook of methods, accompanied by an atlas along with short textual descriptions of the more important organisms found in disease. The work will be especially valuable to those who wish to have a laboratory handbook giving the most recent methods now in use, especially in the German laboratories, in which, from internal evidence, it would seem that the author has comparatively recently been working. His classifications and descriptions of organisms, moreover, are those usually adopted in Germany.

"The author commences with a very sketchy classification of bacteria, followed by a short account of their morphology. He then gives methods of sterilisation, one of which, a very simple one, will be useful to those using *post-mortem* instruments, and may be mentioned here. The knife is dipped in benzine, which is then lighted, the flame being sufficient to sterilise the metal. If this contention be borne out in practice, this method should be a very convenient one, especially for laboratory use. The principles of bacteriological technique, though somewhat condensed, will be specially useful for reference by those who are being, or have been, taught in a practical class. The section dealing with staining methods is very full, and many of the stains given are exceedingly good. . . .

"The most important new features in the part devoted to special bacteriology are that the author gives an account of the bacteria found in milk, whilst he lays considerably more stress on those organisms that are found in diseases of animals than do most authors of bacteriological text-books. He discusses such conditions as African farcy, pleuro-pneumonia, contagiosa bovis, foot-and-mouth disease, canine distemper, gives an account of the streptothrices, and also of the bacteria associated with meat poisoning; whilst for the general student of bacteriology he goes into the causation of acetic and butyric fermentation, deals specially with the abnormal fermentative changes occurring in beer, wine, and sugar, and gives an account of the organisms occurring in the leguminous nodules and in the nitrifying processes. He also has chapters on the hyphomycetes or mould fungi; on the various forms of pathogenic and non-pathogenic blastomycetes or yeasts; whilst in a supplementary chapter he gives an account of the protozoa or animal parasites, of which he draws up a list of a dozen, including those found in the Texas cattle fever, in the Australian tick fever, and in the ixodic fever of Jamaica. . . .

"The work is well printed, and the microphotographs are excellent."—*British Medical Journal*.

“ . . . Thanks to Professor Bowhill, we have ‘A Manual of Bacteriological Technique and Special Bacteriology’ by a Fellow of the R.C.V.S.

“The book is beautifully illustrated by more than a hundred woodcuts and photo-micrographs, all of which, save three or four acknowledged, are the original work of the author from specimens of his own preparation. The work is a trustworthy and handy guide for any student—old or young. Controversial matters are omitted, and a clear account, in a concise style, given of micro-organisms, their classification, morphology, and cultivation. The author claims that ‘the technique and working methods have been carefully selected, and from the mass of available material on this rapidly growing branch of the subject, only those methods and material have been chosen which possess distinctive benefits.’ The claim is well justified.

“The book is divided into Parts and Chapters, and the arrangement followed is to treat the subject in this order:—The Classification and Morphology of Bacteria, Methods of Sterilisation, Principles of Bacteriological Technique, Preparation of Nutrient Media, Methods of Cultivation, Special Bacteriology, The Hyphomycetes or Mould Fungi, the Blastomycetes or Yeast Fungi, Pathogenic Blastomycetes, The Protozoa or Animal Parasites.

“The Section on Special Bacteriology treats of all the organismal diseases of man and animals, and will afford information not only to the worker in the laboratory, but to those who merely desire a book of reference. If we take Tuberculosis as a sample, we find the subject treated in a systematic manner under the following sub-heads—Microscopic Appearances, Spore-formation, Staining Reactions, Biological Characters, Vitality, Pathogenesis, and concluding with special paragraphs on Bovine, Equine, Canine, Porcine, Avian, and Pseudo-Tuberculosis.

“The publishers, Messrs Oliver and Boyd, have done their work well, and we congratulate them and the author on having made a useful and sound addition to veterinary literature.”—*Veterinary Record*.

